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THE EVOLUTIONARY SIGNIFICANCE OF THE OSMOTIC PRESSURE OF THE BLOOD[1]

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THE facts of comparative anatomy, embryology and paleontology form the tripod of evidence on which rests to a great degree the validity of the doctrine of evolution. Accepting the doctrine of evolution as a working hypothesis has resulted in clearing up puzzling problems in the above named departments of biological inquiry. At the present time, more attention is being paid to physiological than to morphological problems. In physiology, the great emphasis is placed on mammalian problems with especial reference to man himself. Now if the mammals are the product of a long process of evolution from simple ancestors, it follows that not only has there been a morphological evolution, but also the present complicated functions of the higher animals have evolved from the simpler processes of primitive ancestral forms. In order to understand the significance of particular physiological facts, we must therefore view the matter in the light of evolution. It is not essential that all needful evidence be at hand to make perfectly clear the significance of the higher physiological activity. Indeed, it is well worth while at times to state clearly any of our problems in the evolutionary form and arrange the evidence accordingly.

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In this way we become aware of the need for information to clear up the question which inevitably arises.

It is commonly known that the blood and body fluids of animals possess a certain osmotic pressure. Life processes are constantly dependent on the passage of materials in and out of cells and differences in the osmotic pressure of substances within and without the cell are held to be one cause of this mutual movement. Variations in the osmotic pressure of the blood and body fluids of animals are not so generally known. In the case of severe hemorrhage it is a common practice to replace the lost blood by a physiological salt solution which has the same osmotic pressure as that of the blood. Formerly a 0.7 per cent. saline solution was used. This is isotonic with amphibian blood. The reason for this was that the fact was first discovered in a study of frog's blood. The saline solution (based on amphibian studies) of the physiological laboratories was considered proper for use in hospitals as well. Later it was found that a 0.9 per cent. saline solution represents more nearly the composition of human blood and this solution is in use at present.

But why does human blood have an osmotic pressure equivalent to that of a 0.9 per cent. saline solution? In order to answer this question we must examine all available data as to the osmotic pressure of the blood and body fluids of animals in general. When this is done it appears in many cases at least as though the osmotic pressure of the blood and body fluids were merely a direct adaptation to the environment. But in other cases this is not so clearly apparent, in fact the osmotic pressure possessed by certain forms shows no evident adaptation to the environment at all. The terrestrial vertebrates illustrate this last condition. It is only when we view the entire question from the standpoint of evolution that the main features of the puzzle become apparent.

It might be well to explain at this point the meaning of osmotic pressure. One gram molecule of hydrogen gas at atmospheric pressure occupies 22.4 liters space, and to confine this gas in a space of one liter would require a

pressure of 22.4 atmospheres. A gram molecule of any other gas under the same conditions has the same pressure. Van't Hoff in his theory of solutions established the fact that a substance in solution behaves as a gas occupying the same volume as the solution and the laws which solutions obey are analogous to those which are followed by gases. Therefore a gram-mol of a substance dissolved in a liter of pure water would have the same pressure as a gram-mol of gas, *i. e.*, 22.4 atmospheres. This pressure property of dissolved substances is called osmotic pressure. Since the blood and body fluids contain salts and other substances in solution, these fluids therefore have a certain osmotic pressure. It is well known that a salt solution has a lower freezing point than that of pure (distilled) water. The difference is proportional to the difference in concentration. Since the osmotic pressure depends on the concentration, it follows that the amount of the depression of the freezing point of the solution below that of distilled water is a measure of the osmotic pressure. The osmotic pressure stated in atmospheres can be readily obtained from the "Δ" or depression of the freezing point by the use of the following formula. Osmotic pressure in atmospheres $= (\Delta \times 22.4)/1.85$.

The blood of a vertebrate serves two double purposes. It carries oxygen to tissues and carbon dioxide away. This is its respiratory function. It also carries nutrients to tissues and wastes of metabolism from tissues. We might call this the nutrient function. But the blood of the earthworm is mainly a respiratory fluid. The body cavity is filled with foods absorbed directly from the intestine and distributed by the peristaltic movements of the body to the various tissues. In insects the air is carried directly to the tissues through tracheæ while a so-called heart lying on the dorsal side of the intestine and open at its anterior and posterior ends aids in churning and distribution of food absorbed into the body cavity from the intestine. The indefiniteness of the term "blood" is at once apparent. Most persons in using this

TABLE I

SHOWING THE FREEZING POINTS (Δ) OF THE BLOOD OF ANIMALS

Species	Δ Blood	Δ Water	Locality	Observer
I. Coelenterate:				
1. *Alcyonium*	2.195	2.29	Naples	Bottazzi
II. Echinodermata:				
2. *Asteropecten*	2.312	2.29	Naples	Bottazzi
3. *Asterias*	2.295	2.29	"	"
4. *Holothuria*	2.315	2.29	"	"
III. Annelida:				
5. *Sipunculus*	2.31	2.29	Naples	Bottazzi
IV. Arthropoda:				
6. *Homarus vulgaris*	2.292	2.29	Naples	Bottazzi
7. *Maja squinada*	2.36	2.29	"	"
8. *Maja verrucosa*	2.13	2.29	"	Fredericq
9. *Homarus americ.*	1.82	1.80	Woods Hole	Garrey
10. " "	1.78	1.76	St. Andrews	Macallum
11. *Limulus*	1.90	1.82	Woods Hole	Garrey
12. "	2.04	?	?	Macallum
13. *Astacus*	0.80	0.03		Fredericq
14. *Barbus*	0.475	0.03		"
V. Mollusca:				
15. *Aplysia*	2.31	2.29	Naples	Bottazzi
16. *Octopus*	2.24	2.29	"	"
VI. Cyclostomata:				
17. *Polistrotema*	1.966	1.924	Monterey	Greene
VII. Elasmobranchii:				
18. *Mustelus vulg.*	2.36	2.29	Naples	Mosso
19. *Trygon viol.*	2.44	2.29	"	"
20. " "	2.378	2.29	"	Bottazzi
21. *Mustelus laev.*	2.36	2.29	"	"
22. *Scyllium stell.*	2.31	2.29	"	"
23. *Torpedo ocell.*	2.351	2.29	"	"
24. *Torp. marmorata*	2.292	2.29	"	"
25. *Squatina angelus*	2.28	2.29	"	"
26. *Acanthias vulg.*	1.90	1.91	North Sea	Dakin
27. *Raia clavata*	1.90	1.91	" "	"
28. *Carcharias lit.*	2.03	1.82	Woods Hole	Garrey
29. *Mustelus canis*	1.88	1.82	" "	"
30. *Mustelus canis*	1.869[2]	1.81	" "	Scott
31. *Squalus acanthias*	1.84	1.81	" "	"
32. " "	1.70	1.42	New York	"
VIII. Pisces:				
33. *Acipenser sturio*	0.76	2.00	Arcachon	Rodier
34. *Charax*	1.040	2.29	Naples	Mosso
35. *Serranus*	1.035	2.29	"	"
36. *Conger vulg.*	1.120	2.29	"	Bottazzi
37. *Deutex vulgaris*	1.022	2.29	"	"
38. *Oncorhynchus*	0.762	1.924	Monterey	Greene
39. *Pleuronectes fles.*	0.883	1.91	North Sea	Dakin
40. *Pleuronectes plat.*	0.71	1.91	" "	"
41. *Lophius*	0.80	2.00	Arcachon	Rodier
42. *Lump sucker*	0.648	1.90	North Sea	Dakin
43. *Gadus mor.*	0.72 (0.64)	1.80	Baltic	Dekhuyzen
44. *Pleuronectes*	0.681	1.80	"	"
45. *Conger vulg.*	0.74	1.80	"	"
46. *Cottus scorp.*	0.941	.....	Amsterdam	"
47. " "	1.178	.....	Helder	"

[2] Mean Δ of eighty specimens.

Species	Δ Blood	Δ Water	Locality	Observer
VIII. Pisces:				
48. *Gadus angel*.....	0.767	1.80	Baltic	"
49. *Gadus virens*.....	0.76	1.80	"	"
50. *Gadus merl*.....	0.86	1.80	"	"
51. *Molva vulg*.....	0.716	1.80	"	"
52. *Molva byrkel*.....	0.86	1.80	"	"
53. *Motella tric*.....	0.605	1.80	"	"
54. *Hippoglossus*.....	0.671	1.80	"	"
55. *Pleuronectes pl*.....	0.672	1.80	"	"
56. *Pleuron. micro*.....	0.681	1.80	"	"
57. *Labrus bergylta*.....	0.694	1.80	"	"
58. *Labrus mixtus*.....	0.681	1.80	"	"
59. *Conger vulg*.....	0.696	1.80	"	"
60. *Salmo trutta*.....	0.785	1.80	"	"
61. *Labrax lupus*.....	0.72	1.80	"	"
62. *Trigla hirundo*.....	0.669	1.80	"	"
63. *Anarrichas*.....	0.665	1.80	"	"
64. *Agonus cataphr*.....	1.095	.....	Helder	"
65. *Zoarces*.....	1.30	.....	"	"
66. *Tautoga onitis*.....	0.86	1.82	Woods Hole	Garrey
67. " "	0.70	1.42	New York	Scott
68. *Cyonoscion*.....	0.792	1.82	Woods Hole	Garrey
69. *Conger eel*.....	0.82	1.82	" "	"
70. *Anguilla*.....	0.90	1.82	" "	"
71. "	0.635	1.91	North Sea	Dakin
72. *Scup*.....	0.75	1.82	Woods Hole	Scott
73. *Morone am*.....	0.735	1.82	" "	"
74. *Oncorhyncus*.....	0.628	0.03	Fresh water	Greene
75. *Morone am*.....	0.571	0.03	" "	(Scott)
76. *Anguilla*.....	0.57	0.03	" "	Dakin
77. *Pleuronectes*.....	0.68	0.03	" "	"
78. *Perca*.....	0.507	0.03	" "	Dekhuyzen
79. *Esox lucius*.....	0.519	0.03	" "	"
80. *Salmo fario*.....	0.567	0.03	" "	"
81. *Abramis blicca*.....	0.497	0.03	" "	"
82. *Cyprinus carpoo*.....	0.527	0.03	" "	"
83. *Tinca vulgaris*.....	0.519	0.03	" "	"
84. *Leuniscus eryth*.....	0.495	0.03	" "	"
85. *Erythrinus*.....	0.577	0.03	" "	"
86. *Abramis brama*.....	0.51	0.03	" "	Dakin
87. *Cyprinus carpio*.....	0.487	0.03	" "	"
IX. Amphibia:				
88. *Rana escul*.....	0.563	.....	.....	Bottazzi
89. *Bufo viridis*.....	0.761	.....	.....	Bottazzi & Ducceschi
90. *Bufo vulgaris*.....	0.445	.....	.....	Bottazzi
X. Reptilia:				
91. *Thallassochelys*.....	0.61	2.29	Naples	Mosso
92. *Emys europa*.....	0.463	2.29	"	Bottazzi & Ducceschi
93. " "	0.440	2.29	"	Bottazzi
XI. Aves:				
94. Capon.....	0.66	.....	.....	D'Errico
95. Turkey.....	0.75	.....	.....	"
96. *Gallus bank*.....	0.623	.....	.....	Bottazzi
XII. Mammalia:				
97. *Delphinus phocæna*...	0.74	1.90	.....	.....
98. Horse.....	0.58	.....	.....	Findlay
99. "	0.565	.....	.....	Winter

Species	Δ Blood	Δ Water	Locality	Observer
XII. Mammalia:				
100. Ox	0.601			Findlay
101. "	0.55			Winter
102. Pig	0.625			Findlay
103. "	0.55			Winter
104. Dog	0.599			Findlay
105. "	0.565			Winter
106. Rabbit	0.578			Findlay
107. "	0.57			Winter
108. "	0.564			Bottazzi & Ducceschi
109. Sheep	0.55			Winter
110. Cat	0.615			Findlay
111. MAN	0.560			"

term think of the fluid circulating in the blood vessels of a vertebrate. The term body fluid is also ambiguous. In an invertebrate it has reference to that part which we call the blood of a vertebrate. In the vertebrate we usually think of the secretion of serous membranes as "body fluid." After all, the subject of discussion in this paper is the fluid by which food is carried to tissues and wastes carried away. Having thus defined the use of the terms, let us examine the osmotic pressures of the blood of various animals.

Table I, which follows, shows one hundred and eleven determinations of the osmotic pressure of the blood of representatives of nearly every animal phylum. Many of these determinations are averages. Some of the forms are wholly terrestrial, some live in fresh water, some in either fresh or seawater, some live wholly in the sea. Considerable variation in the osmotic pressure of the blood is shown.

Of the marine forms given some are found in the Mediterranean, while others for the most part occur in the ocean or in protected waters connected with it. There is great variation in the osmotic pressure of the blood of forms living exclusively in the Mediterranean. Great variation is shown in the case of those living in the ocean. In some cases in each environment, complete harmony with or rather isotonicity with the environment is apparent. In other cases this is not at all evident. For

example, the average Δ of twelve species of invertebrates from the Mediterranean is 2.281°, while the average Δ of the water in which they live is 2.29°. A simple case of adaptation is thus evident. But the bony fishes, teleosts, tell a different story.

It is worth while to contrast the osmotic pressure of the blood with that of the external medium. To do this we will break up all the forms into groups not according to the environment alone, but also according to relationship. If we should be guided by environment alone, the result would be a confused tangle. Table II shows the average Δ of these groups selected not only on the basis of relationship but also taking into partial consideration the environment.

"Δ," Blood,	12 Invertebrates,	Mediterranean	= 2.28°	—Water = 2.29°
"Δ," Blood,	4 Invertebrates,	Ocean, bays	= 1.82	—Water = 1.79
"Δ," Blood,	3 Invertebrates,	Fresh water	= 0.592	—Water = 0.03
"Δ," Blood,	1 Cyclostome,	Ocean, bay	= 1.966	—Water = 1.924
"Δ," Blood,	8 Elasmobranchs,	Mediterranean	= 2.346	—Water = 2.29
"Δ," Blood,	6 Elasmobranchs,	Ocean, bays	= 1.902	—Water = 1.85
"Δ," Blood,	4 Teleosts,	Mediterranean	= 1.054	—Water = 2.29
"Δ," Blood,	32 Teleosts,	Ocean, etc.	= 0.744	—Water = 1.82
"Δ," Blood,	13 Teleosts,	Fresh water	= 0.545	—Water = 0.03
"Δ," Blood,	4 Amphibia,	Fresh water	= 0.551	—
"Δ," Blood,	6 Reptilia,	.....	= 0.56	—
"Δ," Blood,	3 Aves,	.....	= 0.67	—
"Δ," Blood,	8 Mammals,	.....	= 0.577	—

From this table it is evident that the blood of the marine invertebrate is isotonic with the water in which it lives, whether this be the Mediterranean or the ocean. As stated above, it appears to be a simple case of adaptation. But in the other cases the relation is not so simple. If we compare the osmotic pressure of the marine teleosts, fresh-water teleosts and the amphibia, etc., with the osmotic pressure of the external medium great differences are evident. And yet it can not be said but what all these forms are adapted to their environment. But it is not enough to make this statement, but to try to explain why such a relationship becomes possible. The isotonicity existing between the blood of marine invertebrates and

their environment has been discussed by Fredericq ('85–'04), Rodier ('99), Dakin ('08), Garrey ('05) and Bottazzi ('97–'06). Now it is held that evolution of life began in the sea. The single celled forms were completely surrounded by the sea and it is easily understood why the osmotic relations would remain primitive in case of these forms. In gastrula type animals, such as cœlenterata, practically all cells of the body are bathed directly by the sea and as far as we know these forms also are in osmotic equilibrium with sea water. Now with the appearance of mesoderm and a body cavity much of the body is removed from direct contact with the sea. But the complete equilibrium remains. As Quinton ('00) says, the marine invertebrate, though anatomically independent of the sea in many of its organs, yet it is still physiologically open to the sea which in an osmotic sense still ebbs and flows throughout its body.

Protoplasm originating in the sea was built up with certain relationships with sea water, which relationships are still maintained throughout all marine invertebrates. May not the sparsity of fresh-water porifera and cœlenterates and the comparative failure of fresh-water algæ be due to the difficulty of maintaining the integrity of protoplasm when all cells of these forms are so freely bathed by fresh water, the osmotic pressure of which is nearly zero?

Next above the marine invertebrates is a single case of a cyclostome which is in osmotic equilibrium with the surrounding sea water. What the osmotic pressure of the blood of a cyclostome in fresh water is, we have no record. It should be noted here that cyclostomes are now regarded as degenerate fishes and on that account any evidence from these forms as to the higher course of evolution must be treated with care. In the next place we find that eight species of elasmobranchs from the Mediterranean and six from the ocean possess blood which is practically isotonic with the sea water outside. Apparently they do not differ from the marine invertebrates. But it is evident that the osmotic pressure of the blood is slightly

greater than that of the external medium. Furthermore, analysis shows that the osmotic pressure of elasmobranch blood is due to different substances from those which account for the osmotic pressure of the blood of marine invertebrates. Therefore the elasmobranchs belong to a second category. In the third group we will place the marine teleosts. The osmotic pressure of their blood is somewhat less than half that of the medium in which they live. We have the case of four species from the Mediterranean and thirty-two species from the ocean which show this. The osmotic measurements show a decided difference between the blood and the surrounding medium. A decided independence also. In the same group or possibly a fourth group we will place the fresh-water fishes and with these the amphibians, reptiles, birds and mammals. Thirteen species of fresh-water fishes possess blood with an osmotic pressure less than that possessed by the marine teleosts. Let us assume here that the fresh-water fishes were derived from marine ancestors. In becoming acclimated to fresh water, the blood suffered a decrease in its osmotic pressure. Whether this was in direct response to the great decrease in the osmotic pressure of the surrounding medium as compared with sea-water is problematical, but appears probable. The amphibians were derived from the fresh-water teleosts. Some of the amphibians still retain their aquatic habits and structures. They in all probability possess the osmotic pressure of fresh-water fishes. Other amphibia metamorphosed into terrestrial forms, taking with them the osmotic pressures of the blood possessed by their fish-like ancestors. Blood with the same osmotic pressure as that of the fresh-water fishes flows on through the amphibia to the reptilia and on to the birds and mammals. An examination of Table II shows the close similarity between the osmotic pressures of fresh-water fishes, amphibians, reptiles, birds and mammals. According to the above hypothesis, the order of evolution was I. Marine invertebrates, II. Elasmobranchs, III. Marine teleosts,

IV. Fresh-water teleosts, amphibians, reptiles, birds and mammals.

Let us examine each of these groups with regard to their osmotic independence of the external medium. That is, what is the effect of changes in the concentration of the external medium on the osmotic pressure of the blood of these groups.

First, the invertebrates. Let us recall Quinton's statement that marine invertebrates are still physiologically open to the sea. For when the concentration of the external medium is changed, it is found that a change in the osmotic pressure of the blood takes place. Fredericq ('85 and '04) stated that the change in one was followed by an equal change in the other. In a few hours the new equilibrium is established. If the time of sojourn in the modified sea water was small the equilibrium with it was not completely attained. Moreover, all invertebrates did not adapt themselves with the same rapidity to changes in the external medium. On the whole, provided the external change was not too great, it was followed in time by complete equilibrium between the osmotic pressure of the blood and that of the modified sea water. This was true in the case of sea water made dilute by addition of fresh water and sea water made more concentrated by the addition of salt. In other words, the organism possesses no structures which render it independent of the changes in the external medium. There are three structures concerned in these changes. First the integument, second, the intestinal wall and third the gill membranes. With the appearance of gills, the body integument apparently is the first structure to become impermeable. The intestinal wall is the first to show a selective action.

Second, the elasmobranchs. These had been placed by investigators with the marine invertebrates not only because their blood possessed the same osmotic pressure as the external medium, but it was thought that when the external medium was changed, the same changes occurred in the blood of the elasmobranch. I made extensive ex-

periments to test this ('13) and found that when a change was made in the external medium, though considerable change took place in the blood of the dogfish, yet it was considerably less than the external change. In fact it appeared as though the change in the blood was roughly proportional to the change in the external medium (p. 20, Scott, '13). The condition was so marked as to show clearly that the elasmobranch belonged in a category differing from that of the marine invertebrate.

Third, the marine teleost. Much emphasis has been placed upon the claim that these forms are absolutely independent of changes in the external medium. With this claim, I must differ. The following evidence is the basis of this difference of opinion. In the first place Tables I and II show that the blood of teleosts from the Mediterranean has a higher osmotic pressure than that of blood of teleosts from the ocean. There is a corresponding though greater difference in the osmotic pressure of the water. Dakin '08 in a trip from Kiel to Helgoland found that the osmotic pressure of the sea water increased 74 per cent. and that the osmotic pressure of the blood of the plaice showed an increase of 20 per cent. The cod did not show so great a difference, being but 4 per cent.[3] Garrey '05 reported Δ of the blood of the tautog at Woods Hole to be 0.86° while at the New York Aquarium, where the harbor water is much more dilute than at Woods Hole, I found the Δ of tautog blood to be about 0.70°. Therefore it would appear that even blood of the marine teleost is somewhat modified by changes in the external medium. And yet practical independence has been achieved. This is evident from the fact that the marine teleost lives in a medium which has an osmotic pressure over twice as great as that of the blood of the fish.

Macallum ('10) has explained the peculiar osmotic pressure of the blood of marine teleosts as due to their origin from fresh-water teleosts. This is based on morpholog-

[3] On the other hand Dekhuyzen, '05, found a difference of 20 per cent. in the osmotic pressure of cod blood according to the locality from which the fish was taken.

ical evidence of the evolution of the true teleosts from ganoid ancestors from the elasmobranchs through forms similar to the sturgeons and the bow-fins. I doubt very much, however, whether ichthyologists would wish to conclude on this basis that all marine teleosts had their origin from fresh-water forms. In fact certain paleontologists trace the evolution of certain fresh-water teleosts from ancestral marine teleosts. The sea is the home of the preponderating fish population. Here the class of Pisces has found its greatest opportunities for range of movements to escape enemies, in search of food or place of breeding.

Facts concerning the osmotic pressure of the blood of anadromous fishes throw light as to the possible if not probable origin of fresh-water forms. Greene ('04) determined the osmotic pressure of the chinook salmon in Monterey Bay to be 0.76°. On the spawning grounds in fresh water its blood had a Δ of 0.628°, a decrease of 17.6 per cent. Flatfish are known to be somewhat anadromous. Dakin ('08) found the Δ of the flounder, *Pleuronectes flesus,* in the North Sea to be 0.83°, while in the River Elbe in fresh water its blood had a Δ of 0.68°, a decrease of 18 per cent. The same author found that the blood of the eel, *Anguilla,* in fresh water had a Δ of 0.57°, quite similar to that of fresh water fishes. After a day in sea water another specimen had blood with a Δ of 0.745°. Eels taken from seawater had blood with a Δ of 0.634°. Eels from seawater placed in fresh water for three days possessed blood with a Δ of 0.582°, practically the same as for fresh-water forms. At Woods Hole, ignorant of this work of Dakin's, I made observations on the Δ of the blood of the white perch, *Morone americana.* This form can live equally well in salt or fresh water. Taken from the slightly brackish waters of Tashmoo Pond, Marthas Vineyard, Massachusetts, the blood showed a Δ of 0.635°. The upper end of this pond is the source of drinking water for Oak Bluffs. A number of perch were placed in running tap water for a day, when the blood showed a

Δ of 0.571°, similar to the fresh-water fishes. Others of this lot were placed in sea water for two days, when the Δ of their blood was 0.766°. Others taken directly from the Eel Pond (sea water) showed a Δ of 0.735°. The result is similar to Dakin's. On the whole the conclusion seems justified that anadromous fishes are able to adapt themselves to a degree to the great changes in the osmotic pressures of the external medium, which they meet in passing from salt to fresh water or vice versa by a slight corresponding change in the osmotic pressure of the blood.

It is commonly known that sturgeons are anadromous. For some reason the elasmobranch has been shut out of fresh water. There is but one elasmobranch known to inhabit fresh water, *Carcharias nicaraguensis* of certain lakes in Nicaragua. Although the integument of the shark is impermeable, yet I have found the gills to be still permeable to salts (Scott & Denis, '13). The ganoids derived from elasmobranchs ventured up fresh-water streams. They returned to the sea. Rodier ('99) states the Δ of the blood of *Acipenser sturio* to be 0.76°, which places it in the same group as the marine teleosts. What the Δ is in fresh water is not known. The modern sturgeon is a long way from the modern shark. Nevertheless it is conceivable that the ancestral ganoids tried fresh-water conditions. Is it not possible that these conditions, fresh water and food found in fresh water had some influence on the change in structure. During all subsequent periods when evolutionary changes were taking place some forms went back and forth from sea to fresh water. Some forms remained in fresh water. During this period of experimentation, impermeable membranes were built up. In the meantime the blood had become modified, due to the temporary sojourn in fresh water. The osmotic pressure was reduced; the membranes once made practically impermeable remained so, and when those forms returned to the sea and remained there they retained *almost* unmodified the osmotic pres-

sures they had acquired during their fresh-water experience. We can thus speculate that in some such way the present osmotic pressures of the blood of marine and fresh-water teleosts were acquired. Whatever may be the case with the marine and fresh-water teleosts, it is more clearly indicated that the osmotic pressure of the blood of terrestrial forms is derived from fishes which lived in fresh water. The present day anadromous fishes constitute all that remains of a movement which at one time was far more general.

The chemical composition of the blood throws further light on the question. The osmotic pressure is due to substances dissolved in the blood. These are chiefly salts. Quinton ('00) states that sodium chloride represents from 85 per cent. to 90 per cent. of all the dissolved salts of the blood. The sodium chloride content can be ascertained from a study of the chlorides which are easily determined. Let us ascertain the changes in the sodium chloride content of the blood of the forms under discussion. In the first place what is the total salt content of sea water. According to Bottazzi ('97) the total salt content of water from the Mediterranean is 3.78 per cent. The water of the ocean contains about 3.22 per cent. salts. Of course there is some variation. The percentage of salts in fresh water is very small, 0.05 per cent. (Sumner, '05). What is the percentage of salt of the blood of forms living in the sea? Quinton ('00) made forty-nine determinations of the sodium chloride content of the hemolymph of ten species of marine invertebrates belonging to five different groups and found that these averaged 3.24 per cent. He made 26 determinations of the sodium chloride content of the sea water in which these forms lived, and found that it was about 3.31 per cent. According to these researches of Quinton, the blood of the marine invertebrate contains about the same percentage of salts as the water in which they live. Moreover, it follows that the osmotic pressure of the blood is determined almost wholly by the salts of the blood and not by any organic solutes. It was because

of this relationship that Quinton felt justified in making the statement that the marine invertebrate while anatomically closed to the external medium, is yet physiologically open to it. That functionally speaking the marine invertebrate is still freely exposed to the sea without, which still practically ebbs and flows through its body.

Macallum ('10) says:

> In *Limulus,* the amount of total salts in the blood, 2.982 per cent., approaches that of the sea water,—which may be found along the Atlantic coast. At St. Andrews, New Brunswick, the total salts of the seawater collected in April were 2.417 per cent., but in sea water collected in August, 3.165 per cent. In the blood of the lobster, the total salts as ascertained were 2.852 per cent., which is between the two concentrations given above for the salinity of the sea water at St. Andrews where the lobsters from which the blood was taken were obtained. The blood of *Limulus* is but slightly modified sea water. It would appear as if the sodium chloride of sea water passes freely into the blood of the lobster till the sodium chloride concentration in both is approximately balanced.

This agrees entirely with the work of Quinton. For some reason, the marine invertebrate has not been able to keep the sea out. One asks why the question of the permeability of membranes of fishes to salts is of such interest to the comparative physiologist? One answer is that impermeability represents independence of the sea the osmotic pressure of which differs so much from that of fish blood. And this independence is not to be found among the marine invertebrates.

As shown above, elasmobranch blood possesses the same osmotic pressure as that of the marine invertebrate and that of the sea without. But analysis shows that the osmotic pressure of elasmobranch blood is due to entirely different causes. For example, what is the salt content of elasmobranch blood? It should contain about 3.22 per cent. salts in order that its total osmotic pressure be due to salts. But Rodier ('99) found that the blood of elasmobranchs did not contain over 1.7 per cent. sodium chloride. Dakin ('08) found the blood of the dogfish to contain but 1.45 per cent. sodium chloride. My analysis of the blood of another species, *Mustelus,* at Woods Hole

showed 1.424 per cent. sodium chloride. Fredericq ('04) found the blood of *Scyllium* to contain but 1.71 per cent. salts, while Macallum ('10) found the blood of the dogfish, *Acanthias vulgaris,* contained 1.7739 per cent. sodium chloride. In other words the sodium chloride content of the blood of elasmobranchs will account for only about half of its total osmotic pressure. Evidently a great change has come about. "The difference between the Δ of the serum and that due to salts of the serum depends," as Macallum ('10) says,

"on urea and other organic solutes." Urea is present in large quantities in the blood of elasmobranchs.

Staedeler and Frerichs ('58) obtained as much as two ounces from the the liver of a single shark. In '90 von Schroeder found that *Scyllium,* another dogfish, contained blood with 2.6 per cent. urea. Rodier ('99) computed that one third the osmotic pressure of the blood of elasmobranchs was due to urea.

In '13, I found that *Mustelus* blood contained 1.55 per cent. urea. Macallum ('10) in *Acanthias vulgaris* found an average of 2.026 per cent. urea. Due to dissociation, the salts have twice the osmotic pressure, approximately, as the urea, although the urea and salts are present in about equal quantities. But the urea and salts are not sufficient to account for the osmotic pressure of the blood. The difference is due to the presence of ammonia salts, as Macallum found. For example, he found 0.1727 per cent. ammonia in the serum of the dogfish. This would fully account for the remaining percentage of the depression of the freezing point unaccounted for by the presence of the salts and urea. So that we see, that while superficially the elasmobranch resembles the marine invertebrate in the osmotic pressure of the blood, yet below the surface a marked change has taken place. Several observers had noted that the osmotic pressure was slightly greater than that of the sea water. This at least is another indication that the equilibrium is not like that existing between marine invertebrates and the sea. For some reason the elasmobranch has lost in salts. Their place has been taken by *nitrogenous* solutes. The con-

dition is lacking in the marine invertebrate. Some one has characterized the jellyfish as organized sea water. According to Macallum the blood of *Limulus* is but slightly modified sea water. The blood of the marine invertebrate has remained at the same low level so far as the presence of nitrogenous compounds is concerned. To what may we ascribe this new condition? Is it due to great proportion of nitrogenous food? To the particular kind of liver? To the great development of the muscular system? To a peculiar function of the kidney? Questions can at present be asked only. We lack information as to certain aspects of elasmobranch physiology.

However much the elasmobranch may have experimented in the matter of unique nitrogenous content of the blood, it is certain that this condition is lacking in the teleosts. And the lack there is carried over to the forms which developed further. For the osmotic pressure of the blood of teleosts is again determined almost wholly by the salts present. The salt content of the blood of marine teleosts is considerably less than that of elasmobranchs. Quinton ('00) found the blood of eight species of marine teleosts to contain 1.076 per cent. salts. Rodier ('99) found that the blood of the ganoid, *Acipenser sturio,* had a salt content varying from 0.643 per cent. to 0.979 per cent. The blood of *Lophius,* a strictly marine form, contained 1.164 per cent. salts. Hamburger states that teleost blood contains 0.936 per cent. salts, but whether these are fresh-water or marine species is not stated. Macallum ('10) found that the blood of the cod, *Gadus callarias,* contained 1.2823 per cent., while that of the pollock, *Pollachius virens,* contained 1.2934 per cent. salts. It is evident, therefore, that the percentage of salts in the blood of the marine teleost has been decreased as compared with the total saline content of elasmobranch blood. Moreover, the osmotic pressure of the blood of the teleost is due almost wholly to the salts present. Macallum ('10) proved this. He found that the Δ of the salts of cod blood was 0.71°, while that of the entire blood was 0.765°.

There is a difference of but 0.055°. The Δ of the salts of the blood of the pollock was 0.737° while the Δ of the entire blood was 0.825, showing a difference of but 0.088°. In other words, almost the entire osmotic pressure of the blood of the teleost is due to the salts. The urea, ammonia or other organic solutes present must be very small and are represented by the difference above mentioned, namely 0.055° in the case of the cod and 0.098 in the case of the pollock. How different is this condition from that found in the elasmobranch where in one case noted by Macallum, and which is typical, the difference between the Δ of the saline contents of the blood and the entire blood was 0.961°, a difference as great as the average Δ of the marine teleost and as stated due to the relatively enormous amount of urea and other organic solutes in the blood of the dogfish. Again the question arises: What brought about this change between the composition of elasmobranch blood and that of the teleost? Was it due to the migrations to and from fresh water before certain species of teleosts took up their home permanently in the sea? And yet the marked difference between the two is not alone a difference in salt content. It is far more the absence from the blood of urea, ammonia and other organic solutes. Let us use Macallum's data as a basis for comparison. The blood of marine teleosts contains about 30 per cent. less salts than the blood of elasmobranchs but it contains 90 per cent. less organic solutes. The distinct loss therefor is in organic solutes. This therefore must have been a significant factor in the evolution of the higher form. Now what is the most apparent structural difference between the elasmobranchs and teleosts? It is of course that the skeleton of one consists of cartilage and the skeleton of the other is bone? It does not necessarily follow, however, that the power to build a bony skeleton depends on the absence of organic solutes from the blood, nor is there apparently any close connection between them.

The fresh-water fishes in all probability agree with the

marine teleosts in low percentage of organic solutes and this characteristic is maintained by all the higher forms. Dakin found that the blood of the plaice at Helgoland contained 0.92 per cent. salts, while at Kiel in brackish water it had a salt content of 0.85 per cent. Mosso ('90) stated that marine teleost blood had a higher salt content than that of fresh-water forms. Dakin ('08) found the blood of the eel in sea water to contain 0.605 per cent. salts, while in fresh water its saline content was 0.466 per cent. Quinton ('00) found that the blood of fresh-water teleosts contained 0.7 per cent. salts. Atwater ('91) found that the flesh of fresh-water teleosts contains less salt (15 per cent. less chlorine) than that of marine teleosts. Sumner ('05) obtained a similar result.

The anadromous fishes possess blood that is less saline in fresh water than in sea water. It is also true that strictly marine teleosts of the present day vary a little in the saline content of their blood when the salinity of the external medium changes. These facts indicate that the decreased salinity of the blood of fresh-water teleosts was brought about in response to the low saline content of the external medium. During the migrations that took place in the past when there were probably more anadromous fishes, this diminution in salts took place. Those forms that remained in fresh water retained the percentage of salts they acquired by their sojourn in fresh water. At the same time they built up membranes which maintain an equilibrium in spite of the differences in the osmotic pressure of the blood within and the fresh water without. Similar membranes were formed in case of the marine teleosts, which maintain an equilibrium with the sea water in spite of the fact that the osmotic pressure of sea water is over twice that of the teleost blood. The evidence at hand indicates that the last membranes to become practically impermeable to salts were the gill membranes. And yet though impermeable to salts they still are required to be permeable to gases.

Now the blood of amphibia contains about 0.7 per cent.

salts. This closely resembles that of fresh-water fishes. The blood of mammals contains a slight increase in its saline content. Bunge ('89) states that human blood serum contains about 0.84 per cent. to 0.86 per cent. salts. Macallum ('10) calculating from Abderhalden's analyses, concluded the total saline content of the blood of the dog amounted to 0.935 per cent., that of the cat to 0.933 per cent. and that of the sheep to 0.905 per cent. To quote from Macallum:

> In mammals, according to Abderhalden's analyses, there is an extraordinary similarity in the inorganic composition of the serum of the number of the forms taken and the ratios of the sodium, potassium, calcium, and magnesium are almost parallel with those in the Teleosts and Elasmobranchs.

Macallum had an opportunity to analyze the blood of "the whale common in the Pacific off the coast of British Columbia," and the parallelism between the inorganic constituents of its blood and that of the horse and pig was remarkable, thus bringing the whales very close to the Ungulates to which some anatomists relate them.

The above studies of the osmotic pressures of the blood, the change in the permeability of the protecting membranes and the inorganic and organic composition of the blood are understood only by viewing them from the standpoint of evolution. The increase in saline content of mammalian blood as compared with amphibian and fresh-water teleosts can be ascribed to the regulative action of the kidney. Most investigators give the impression that the osmotic pressure of the blood of animals is definite and fixed. This is not true. Findlay calls attention to the variation in the osmotic pressure of human blood at different times of day. For example, a distinct though slight rise (0.03°) is noted after meals. This question needs further study. My investigations showed that *Mustelus canis* can pass with entire safety through a range of 0.15° (+ and −) in its osmotic pressure. The range through which invertebrates can pass is much greater. The observations of Dekhuyzen ('05) and Dakin ('08) show that the range becomes limited in the case of

marine teleosts. The range is very much more restricted in fresh-water teleosts and higher forms. Protoplasm is an ever-changing substance. There is a constant ebb and flow. Protoplasm of the higher forms has evolved through long ages to a condition wherein it is associated with the same salts it was entirely surrounded by when it first began to be. The amounts have changed, but the proportions have remained quite constant and the kinds have remained the same as those in the sea. And that is why the surgeon must inject a 0.9 per cent. saline solution into the veins of his patient suffering from hemorrhage. And that is why human blood has a certain osmotic pressure. Macallum ascribes the first great reduction in salts which took place in the elasmobranch to be due to the kidney, whose primary function was not the elimination of the wastes of metabolism, but the regulation of the concentration and composition of the salts of the blood. The elasmobranch kidney is very inert and sluggish in the matter of the elimination of the organic wastes. The teleosts acquired the habit of still further keeping down the saline content while at the same time they eliminated the urea readily. However, I do not see that the process is necessarily limited to the kidneys alone. A thorough study of the elasmobranchs and teleosts is needed to throw light on this puzzle. I can see why the migratory habits of teleosts or teleost ancestors (ganoids) would account for reduction in salt content of the blood, but this throws no light on the reduction of salts in elasmobranch blood as compared with invertebrate blood. Nor does Macallum indicate any use the large amount of urea might serve. Balgioni ('06) found that salt solution alone led to stoppage of the elasmobranch ventricle in diastole. It increased diastolic tonus, while urea increased systolic tonus. The presence of the two in about equal amounts mutually neutralized each other and made the continuous rhythm of the heart possible. All we can say is that for the kind of protoplasm of which the elasmobranch heart is composed, the urea is a necessary constituent of the blood. Furthermore it does not appear to be necessary

for the teleost heart. At any rate we are aware that once we begin to question further, the necessity of further knowledge becomes evident. This paper can be brought to a close in no better way than by quoting a statement made by Claude Bernard ('65). We may accept it as one of the laws of evolution and conclude that inquiries concerning the osmotic pressures of the blood of animals amply prove its truth.

Chez tous les êtres vivants, le mileau intérieur, qui est un produit de l'organisme, conserve des rapports nécessaires d'échanges et d'équilibre avec le mileau cosmique extérieur; mais, à mesure que l'organisme devient plus parfait, le mileau organique se spécifie et s'isole en quelque sorte de plus en plus du milieu ambiant.

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THE GENETIC BEHAVIOR OF MICE OF THE COLOR VARIETIES "BLACK-AND-TAN" AND "RED"

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EARLY in 1914 there were received at the Bussey Institution certain stocks of mice obtained from fanciers in England. Some preliminary studies of the mice were made by Professor Castle and Dr. Little. A more intensive study of one race, the black-eyed-white, was afterward made by Dr. Little and independently by Dr. Detlefsen. The remaining stocks were turned over to Mr. W. F. Whittier, who carried on experiments with them partly at the Bussey Institution, partly at the Massachusetts Agricultural College, recording some 2,500 offspring. After devising the color grading scale and the general methods followed in the later experiments, he relinquished the work to the present writer. Since that time about 2,000 young have been recorded, bringing the total to 4,500. All the work has been done under the advice and direction of Professor Castle.

The principal varieties which have been used in these experiments are known in England as "black-and-tans" and "reds." The genetic character of these mice was at the outset quite unknown, and in this paper it is proposed to give some account of their genetic behavior, and since they have proved to be forms of yellow mice, to assign to them and their derivatives places in a scheme of classification of the yellow varieties.

The black-and-tan race has presented throughout the more interesting problem. In appearance these mice are of an intense shiny black dorsally, with a belly superficially clear yellow. The belly hairs, however, are marked by having dull black bases, hidden by the longer and over-

lying yellow areas of the hairs. Yellow-ticked hairs are occasionally seen on flanks and head, encroaching on the black pigmented parts. This peculiarity increases somewhat with age, but never to such an extent as to make the body color predominantly yellow.

When bred *inter se,* they have been found invariably to be heterozygous, no homozygous black-and-tan having been discovered among a dozen individuals tested by suitable matings. As recessives they have given all-black mice more intense than any we have seen derived from other sources. Forty-two matings *inter se* of pure-bred black-and-tan parents produced 148 young, an average of 3.52 to a litter. Of these young 93 have been black-and-tan and 55 black, a ratio of 1.69:1. This approximates a 2:1 ratio more closely than the 3:1 ratio usually given by Mendelian heterozygotes. The black recessives breed true, and when mated to black-and-tans have produced equal numbers of black-and-tan and black young (18:18). The approximation of a 2:1 ratio in matings of black-and-tans *inter se* shows their gametic similarity to yellow mice whose unfixable nature was first shown by Cuénot ('03). Figures given by this author combined with those given by Castle and Little ('10), by Little ('10 and '11) and by Miss Durham ('11) total 2,673 young produced by yellow $\times$ yellow matings. Of these 1,783 were yellow and 890 non-yellow, a ratio of almost exactly 2:1.

The small average size of litters produced by black-and-tan parents mated *inter se* gives added evidence of their resemblance to yellow. Castle and Little ('10), in confirmation of Cuénot's observations, showed that yellow $\times$ yellow matings produced litters of smaller average size (4.71) than yellow $\times$ non-yellow (5.57), and following Cuénot they attributed the difference to absence of homozygous, yellow-yellow zygotes. The 2:1 ratio and the small-sized litters serve also to relate the black-and-tans with Castle's "sooties" and Miss Durham's "sables," both of which were shown to be heterozygous yellows carrying black as a recessive.

The reds, by their appearance, gave promise of being some form of yellow. The color, as the name implies, is orange-red dorsally, the belly being a lighter shade. Up to the age of three weeks the young mice are dusky yellow-red, the red apparently being obscured by a darker pigment. As they grow older they become progressively of a brighter and more intense reddish hue.

Genetically these mice behave much like black-and-tans. None has been found which has bred true, and the relation of reds to non-red recessives is in the same approximate ratio of 2:1. The recessives in this case are "chocolate," in color a deep, rich brown, showing an intensity comparable to that of the blacks derived from the black-and-tans. Thirty-one matings of red with red have produced a total of 136 young, of which 77 have been red, and 59 brown, a ratio of 1.30:1. The average size of these litters was 4.40. Eleven matings of red with brown produced 34 red and 31 brown young (equality expected), the average size of litters here being 5.90.

So far we have dealt only with the pure stocks, each of which is fairly uniform, although small fluctuations in density of pigmentation do occur. When, however, these two sorts are crossed with each other, yellow mice of various shades are obtained, which form two graded series, roughly parallel, one bearing black pigment and producing black recessives; the other bearing brown pigment and producing brown recessives. Classification in these two series is complicated by the fact that juvenile colors are not uniformly retained, but in some cases increase and in other cases decrease in intensity when the fur is moulted. All animals have therefore been assigned a numerical color-grade at the age of three weeks, this age having been determined as the time when the relation of yellow to black or brown pigment is most definitely visible; and although many animals have been re-graded at intervals throughout life, each has been designated by his original grade.

The cross of black-and-tan with red produced in F_1 two

classes of young. (1) One of these may be described as a black-and-tan in which the black pigmentation is lessened in amount and intensity, this decrease being attended by an increased development of yellow pigmentation. This class closely resembles the variety known as sable. (2) The other class of young consisted of blacks, which also were less intensely pigmented than the recessives produced by pure-bred black-and-tans mated *inter se*. It was found convenient in classifying the young of later generations to recognize six arbitrary grades of blackness of which yellow (showing no black pigment) forms grade 1, and black-and-tan grade 6. On this scale the mean of the F_1 "sable" young was close to 3.5, the intermediate point between yellow and black-and-tan. The distribution can be plotted by translating the descriptive terms in Mr. Whittier's notes into terms of numerical grades, as follows:

Grade	3,	4,	5,	6
Frequency	9,	0,	6,	1

These descriptive notes were made before the grading scale had been adopted, and it is quite probable that no real discontinuity in the variation occurred as would be suggested by entire absence of animals of grade 4. No such discontinuity is found in the work done since the grading scale was adopted.

The F_1 black young were mated *inter se* and back-crossed with browns to test their gametic composition. When mated *inter se* they gave 28 black and 11 brown young, nearly a 3:1 ratio (29:10). Back-crossed with browns they gave 37 blacks and 33 brown young, nearly a 1:1 ratio (35:35). F_1 blacks apparently, then, were simple heterozygotes, not differing from ordinary heterozygotes produced by crossing homozygous black with homozygous brown.

Thirteen of the F_1 sables were tested by mating with browns. One hundred and thirty-three young resulted, of which 70 were yellows of various shades and 63 non-yellows. Of this latter group 32 were black and 21 were

brown, equality being expected. The yellows also may be divided into two groups, in one of which the eyes and fur contain black pigment, while in the other the corresponding parts contain brown pigment. In both of these yellow groups the amount of black or brown pigment varied. Again translating Mr. Whittier's descriptions into terms of the numerical scale, we have the following distribution:

	Grade...	1,	2,	3,	4,	5,	6,	Total
(1) The black series—Frequency...		0,	4,	8,	3,	8,	2 (?),	25
(2) The brown series—Frequency..		13,	1,	4,	16,	1,	2,	37

It was frequently found to be impossible to determine by inspection alone whether a particular yellow animal belonged to the black or the brown series, because yellow fur containing a small amount of black pigment closely resembles that which contains a considerable amount of brown pigment. Consequently these back-cross young (produced by an F_1 sable mated with brown) had to be tested themselves, either by *inter se* matings or by crossing with browns. The classification of the back-cross young in the above tables is based partially on breeding tests and in the cases where these were lacking classification is based on inspection at the age of three weeks. It is uncertain whether any individuals were obtained from the F_1 sable $\times$ brown cross which showed the full intensity of pure-bred black-and-tans (grade 6), although two animals are recorded in the notes as black-and-tan without qualifying terms.

As a result of back-crossing with browns the F_1 sables (out of black-and-tan $\times$ red) and testing the young produced by crossing them with browns, two graded series of yellow mice may be recognized as follows.

Black Series			Brown Series		
Grade	Designation	Producing as Recessives	Grade	Designation	Producing as Recessives
6	Black-and-tan	Black	6	Brown-and-tan	Brown
3–5	Black-sable	Black	3–5	Brown-sable	Brown
2	Sooty yellow	Black	2	Red	Brown
1	Yellow	Black	1	Yellow	Brown

The brown-and-tan and brown-sable varieties are new. They resemble black-and-tan and black-sables, respectively, in which all black pigment in the fur has been replaced by brown pigment. The parallelism between the two series is strongest at top and bottom; red has no exact counterpart in the black series, since its yellow is more intense than that of sable. All members of the two series when crossed *inter se* fluctuate about their parental mean grade. The greatest fluctuations are noted among the offspring of sables; the least among black-and-tans and reds. We suspect also that a like gradation occurs in the amount and intensity of black and brown pigments in the black and the brown recessives of these series, though on account of the self color of these varieties this point is difficult of verification, except by breeding tests. From some tests which have been made and others which are under way, the evidence seems to show that blacks from sables and yellows have less intense young when crossed with agoutis, than do the blacks out of pure black-and-tan. Tables and curves for this cross will be given at a later time.

It is significant now that sables and black-and-tans may be synthesized by a cross of blacks out of the black-and-tan race with reds, showing that the black recessives carry the same differentiating element as do the black-and-tans. Such a cross produced 45 young, 20 of which were black-and-tan or sable, while 25 were black. The F_1 blacks were heterozygous for brown, *inter se* matings producing 32 blacks and 13 browns.

When a black which was heterozygous for brown was mated to a red, yellows falling in both the black and the brown series were produced as follows:

Black Series

Grade	1,	2,	3,	4,	5,	Blk.,	Total
Frequency	1,	1,	2,	0,	2,	1,	7

Brown Series

Grade	1,	2,	3,	4,	5,	Br.,	Total
Frequency	1,	2,	0,	3,	1,	6,	13

The element added in this last cross is plainly the brown gamete carried by the black. This brown gamete, however, has received something additional from the black-and-tan race, so that when red unites with this changed brown gamete the result is a darkening and intensification of the brown pigments to produce a brown-and-tan or brown-sable, a process quite parallel to that which produces black-and-tan and black-sable in the pure black × red cross.

A few crosses were made between pure-bred black-and-tan and brown, and although the numbers here are small, the indication is that the result will be the same as in the black × red cross. F_1 consisted of blacks and black-sables; the sables when back-crossed to browns gave approximately equal numbers of blacks (26) and browns (20) and also the two yellow series as follows:

Black sables (mean grade 3.5)	11	Brown sables (mean grade 4)	10
Yellows and sooties	3	Reds and yellows	10
Total	14	Total	20

This back-cross with the recessive brown gives a direct index of the yellow gametes of the F_1 sables. That they vary in darkness should be borne in mind during the discussion of the difference between black-and-tan and yellow.

The reds in suitable crosses showed the same tendency to produce fluctuating blends. Mated with creams they gave yellows of an intermediate shade (16) and recessive non-yellows (10). These light reds were bred *inter se* and tested by crossing with browns. The young (100 in number) fluctuated in intensity about the shade of the light red parent or parents. Full intensity was not recovered except in back-crosses. Hence red is likewise a form of yellow, differing from it by an added intensity which blends in crosses.

The foregoing evidence has merely pointed to the yellow nature of black-and-tan and red; has classified them and their derivatives among the yellows, and has hinted

at the possible difference between these forms and ordinary yellows. It is time now to inquire as to the real genetic nature of these mice, and to attempt a preliminary explanation of their differences from yellow. By far the largest number of mice have been bred and are being bred toward this end.

Let us consider first the black-and-tan variation. By its behavior it evidently forms two sorts of gametes, black-and-tan (yellow) and black. Each of these has an added something which makes the zygote into whose composition it enters darker than ordinary yellow or black. We may call this something "darkener"—be it singular or plural—and indicate the gametes by YD and BD. Red, similarly, forms gametes red (yellow) and brown; and these also show an addition which we may call "intensifier." The gametes of red are then YI and bI. The sables produced by red × black-and-tan can only be referable to a union of YD and bI, or YI and BD since YDYI is non-viable, and since YDbI and YIBD unions have been demonstrated in the brown × black-and-tan and red × black crosses, respectively, and have produced in both of these latter cases similar sables. The presence of the darkener and the intensifier in the same zygote weakens both and demonstrates their physiological and genetic independence.

The next point to be noted is that both darkener and intensifier are variable. All gametes formed by zygotes containing D or I are not equivalent in their D or I content. It is possible to demonstrate this for the darkener; the variable intensification from crosses with red cannot yet be as satisfactorily shown on account of the difficulties of grading. For light on the action of the darkener we may turn to the agouti crosses.

The ordinary wild house-mouse when bred pure, shows the agouti pattern and gray color with great uniformity. It possesses the black and yellow pigments of the black-and-tan mouse as well as brown pigment, but contains no factor to dilute or darken these pigments. These facts make it an ideal race with which to test for a suspected

darkener which acts on the black pigment of a yellow mouse.

Yellow, Cuénot showed, is an allelomorph of agouti and non-agouti. Black-and-tan, in turn, is not an alternative form of agouti like the light-bellied gray mouse, but a yellow, and hence should be allelomorphic to agouti. And such it is as far as its yellow component is concerned, but not as regards its darkener. The F_1 young from a cross of black-and-tan by wild agouti vary in darkness about a mode midway between black-and-tan and agouti. Black-and-tan we may regard as full darkness and assign to it an arbitrary grade of 6. Wild agouti we may regard as entire absence of this darkness and assign to it the grade 1. F_1 from a cross of these two has consisted of two sorts of young. (1) The first sort may be considered as the result of a union of the YD gamete from black-and-tan with the agouti gamete. These mice have been called sable agoutis; since they have the general pattern of sables. The bellies are yellow; the darkness of the dorsal hairs is variable through the same range as that of the sables, while all hairs on flanks, head and parts of the back are agouti ticked. These yellow F_1 young graded on the sable scale show the following distribution:

Grade	2,	3,	4,	5,	Total
Frequency	1,	15,	7,	4,	27

(2) The second sort of F_1 young may be called non-yellow and referred to a union of the BD gamete from black-and-tan with the agouti gamete. These are simply much-darkened agoutis. The belly is gray like the wild agouti and the flanks are agouti ticked. The head and middle of the back are covered by hairs which are black for most of their length, a very narrow yellow band being present near the tip, or in some cases lacking entirely in an area of hairs down the center of the back. This type is known as dark agouti and has also been graded according to darkness on a scale parallel but not exactly equiva-

lent with the sable scale. Grade 1 was taken as ordinary wild agouti; grade 6 was taken as a gray-bellied black in which the agouti pattern had been lost and in which the darkness was equivalent roughly to that of the black-and-tan. In grade 2 the extent of black in each hair is increased, and the wide yellow band diminished; in grade 3 the yellow is left only in a narrow band; in grade 4 the yellow ticking is lost from hairs in a streak down the center of the back and in grade 5 the area of all black hairs is extended to cover the whole back, ticking being limited to the sides of the body. On the basis of such a scale the F_1 dark agoutis were distributed as follows:

Grade	2,	3,	4,	Total
Frequency	8,	23,	5,	36

These F_1 dark agoutis bred *inter se* have produced 155 young, of which 109 have been dark agouti and 46 black, indicating that the F_1 dark agoutis were heterozygous for black. The distribution of 58 of these F_2 dark agoutis is as follows:

Grade	1,	2,	3,	4,	5,	Total	Mean Grade
Frequency	17,	21,	11,	7,	2,	58	2.2

By using as parents the darkest of these agoutis, regardless of generation, dark agoutis were obtained, which when three weeks old approximated the grade 6. They resemble all-black mice with gray bellies except for occasional ticked hairs on their flanks.

It will be remembered that all darkness in these dark agoutis was acquired originally from the BD gamete of a black-and-tan mouse, since the range of darkness in the wild agouti used has never been above grade 1. Careful grading of the young from matings among dark agoutis should then furnish information as to the variation in the "darkener." If the "darkener" is a multiple thing such matings should afford it opportunity to segregate or Mendelize. A tabulation of matings among all classes of dark

agoutis of the young born since the introduction of the grading scale follows:

DISTRIBUTION BY GRADE OF YOUNG PRODUCED BY DARK AGOUTI PARENTS OF VARIOUS GRADES

Parents	Grade Distribution of Young									
	1	2	3	4	5	5.5	6	Total Agouti	Mean Grade	Black Young
1 × 1	32							32	1.00	1
2 × 2	11	21						32	1.65	1
4 × 2	10	17	5	2				34	2.00	1
4 × 3			6	5				11	3.45	1
4 × 4			6	7				13	3.46	0
5 × 4		1	11	8	18	4	4	46	4.41	14
5 × 5		1	11	15	11	9	4	51	4.34	8
6 × 4		1	4	15	13	4	9	46	4.28	2
6 × 5			4	11	11	18	7	51	4.94	12
6 × 6				5	11	10	11	37	5.29	11
$F_1 \times F_1$ (3 × 3)[1]	17	21	11	7	2			58	2.20	22
6 × 1[2]	5	23	8	1	1			38	2.20	0

It can be seen from a glance at this table that the variation in amount of darkness is a continuous one, from a gray-bellied agouti dorsally all-black, through every possible gradation to a wild-type segregate. The continuous nature of the variation was noted throughout the grading of the dark agoutis in the ever-present tendency to create more classes for the young by adding half and even quarter grades, a temptation which was yielded to only in the grade 5.5, this grade being given to dark agoutis which showed many agouti hairs on shoulders and legs. There is nowhere any evidence of a simple unit-difference between wild agouti and the darkener derived from black-and-tan. The only segregation is that seen in grade 1 animals which bred true and gave no evidence of possessing the darkener.

The above statements are not intended to be final. To date the evidence indicates the presence of a continuously variable and non-Mendelian character which gives the appearance of a blend in cross-bred young, and which in the pure black-and-tan race has been added to yellows carry-

[1] From the cross black-and-tan × wild.

[2] Highest grade dark agouti × wild.

ing black and has darkened and increased their black pigment to the greatest possible extent. The evidence has failed to exclude wholly the possibility of interpreting the darkness of black-and-tan by multiple factors, but the continous nature of the variation in hybrid young would call for the postulation of such a large number of modifiers that this view could be neither proved nor disproved. The nature of the darkener is still to be determined, but its action on both agouti and non-agouti young seems to be to increase the total amount of black or brown pigment produced.

The experiments with the red race do not admit of as definite conclusions as were reached concerning black-and-tan, because of the difficulties of grading for intensity. It is safe to say, however, that red is a race in which yellow has been greatly intensified by a process similar to, though distinct from, that which has produced the darkness of the black-and-tan.

STATISTICAL WEIGHTING FOR AGE OF ADVANCED REGISTRY COWS

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ANY study of milk production that is made from a statistical standpoint must necessarily be complicated, for the reason that advancing age in a cow up to the time she is mature enables her to produce more milk and butter fat. A further difficulty lies in the fact that after maturity the effect of age on production has not been determined with any degree of certainty. Whether or not the increase in capacity is directly in proportion to the advance in age; at what age is the maximum of production reached; what relation is there between age and per cent. of fat in milk, and at what age is a cow past the power of full productiveness, are all questions that need investigation in a broad way.

Necessarily, the various breed associations must have made some comprehensive investigations to enable them to fix standards for milk and fat production, and since the only extensive authenticated records that we have are records of these associations, this study was made for the purpose of determining if their records were consistent with their standards, and if these standards could be used as a basis for weighting cows of different ages.

METHOD OF COLLECTING DATA

Seven-day records only were used, these being secured from the Holstein-Friesian Blue Book, Vol. 24. For the purpose of future investigation all the animals in two direct lines of descent were tabulated, one from a female. the other from a male, both animals being noted ones in the breed. The names, herd book numbers, ages at time

of record, pounds of milk, pounds of fat, and per cent. of fat were all tabulated. Each animal was given an arbitrary number which denoted its position in the generation, and the position of all its direct ancestors in their respective generations back to the primary ancestor of the population. All advanced registry males were tabulated also and numbered.

Records Obtained

From the female, Aaggie Grace, No. 2618, H.H.B., only 456 advanced registry records were obtained in 10 generations. In order to secure these records about twice as many animals were tabulated, the others consisting of the A.R.O. sires and their daughters that had not themselves made A.R.O. records but had two or more A.R.O daughters.

The male, Paul De Kol, No. 14634, H.F.H.B., in 9 generations produced 9,639 female progeny with A.R.O. records. About twice this number of animals were tabulated to secure these records.

Tabulation of Data

Necessarily, before this large accumulation of data could be studied systematically, it was necessary to tabulate it in concise form, and for this purpose correlation tables were made for each population, each table involving a pair of variables. Thus age was compared to pounds of milk, age to pounds of fat, and age to percentage of fat; three tables to each population. From these tables the means of the characters in classes, class average deviations, population means, average and standard deviations and correlation coefficients were worked out. Then from these data, curves were drawn to illustrate its trend graphically.

Results

The correlation tables I and II, compiled for the purpose of studying the frequencies and distributions of the population originating in the male, Paul De Kol, are not

shown here. The one and one and one half year class and the classes over ten years of age were small. For this reason unbalanced and irregular results would be expected for these classes, and by referring to the curves it will be seen that the premise was justified. The two and three year classes were represented by 1,690 and 1,346 individuals, respectively.

Table III gives the average deviations, mean pounds of milk, standard deviations, correlation coefficients and regression coefficients of the population with respect to age and pounds of milk and pounds of fat. Although the mean age is four years, the three and one half year class actually reached the mean pounds of milk of the population, as can be seen from Table IV. Correlation probably amounting to causation is shown in the tables up to six years of age, and after that age is reached the correlation is practically zero.

TABLE III

	Correlation of Pounds of Milk to Age	Correlation of Pounds of Fat to Age
Average deviation	69.8	2.91
Standard deviation	92.4 ± 0.449	3.65 ± 0.018
Mean pounds	395.5 ± 0.635	14.00 ± 0.025
Mean age	4.0 ± 0.013	4.0 ± 0.009
Correlation coefficient	0.604 ± 0.004	0.57 ± 0.005
Regression weight to age	29.84 ± 0.0006	1.11 ± 0.00003
Regression age to weight	0.012	0.29
Coefficient of variability C	23.4 ± 0.001	26.0 ± 0.001

Table IV. This table gives the means, average deviations, and plus deviations of the different age classes for both milk and fat production. From these tables the curves for milk and fat production were plotted. They formed also the basis for calculating the curve which is used as a comparison with the Holstein-Friesian curve of fat and milk requirement. These tables also afford an interesting study from the standpoint of capacity of cows for milk production at different ages.

Considering first the curves for milk production (Fig. 1) it will be noted that curve 1, which represents the

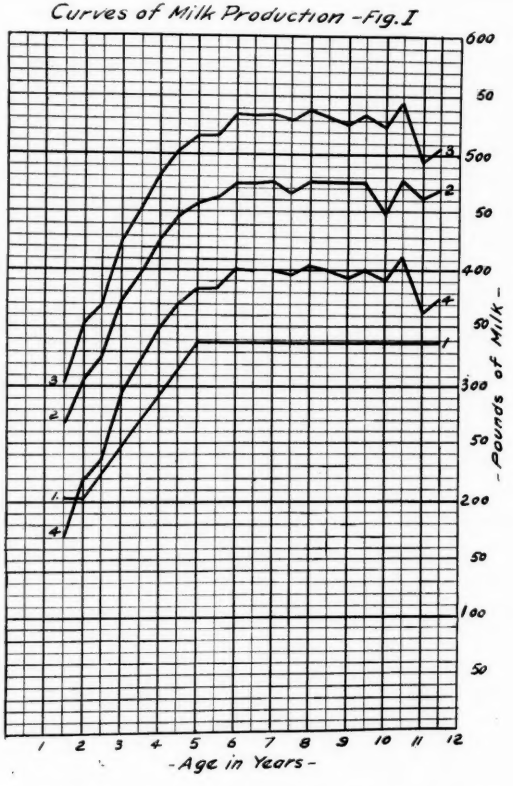
pounds of milk required by the Holstein-Friesian Association, must be calculated from the pounds of fat required. This was done by taking the average per cent. of the whole population and calculating the number of

TABLE IV

Age, Years	Milk Production				Fat Production			
	Means	Av. Dev.	+ Dev.	Curve 4	Means	Av. Dev.	+ Dev.	Curve 4
1½	268	34	302	170	8.9	1.3	10.2	4.76
2	308	44	352	220	10.7	1.74	12.44	7.00
2½	326	43	369	237	11.3	1.76	13.06	7.62
3	372	56	428	296	13.0	2.14	15.14	9.70
3½	396	55	451	319	12.8	2.37	15.17	9.73
4	428	54	482	350	15.2	2.48	17.68	12.24
4½	446	57	503	371	15.5	2.3	17.80	12.36
5	458	59	517	385	16.4	2.36	18.76	13.30
5½	461	56	517	385	16.4	2.3	18.7	13.24
6	474	62	536	404	17.0	2.55	19.55	14.09
6½	474	60	534	402	16.8	2.43	19.23	13.77
7	476	60	536	404	16.7	2.3	19.00	13.54
7½	466	64	530	398	16.8	2.69	19.49	14.03
8	475	65	540	408	17.0	2.54	19.54	14.08
8½	476	58	534	402	16.6	2.46	19.06	13.60
9	476	51	527	395	17.1	2.43	19.53	14.07
9½	475	60	535	403	17.0	2.48	19.48	14.03
10	449	76	525	393	16.6	2.7	19.30	13.85
10½	477	68	545	412	16.7	3.1	19.80	14.33
11	461	35	496	364	16.0	2.0	18.00	12.53
11½	470	36	506	374	15.7	1.43	17.13	11.66
12								

pounds of milk, having the average per cent. that would be necessary to make the required number of pounds of fat. The reason for using the average per cent. of fat of the whole population as a basis for calculating the Holstein-Friesian Association requirement curve was that since the correlation coefficient between age and per cent. of fat was so small in a table shown subsequently for another population, and since the popular concept is that per cent. of fat is not influenced by age, we felt justified in using it. Attention is called to Table V, which does not bear out this assumption entirely. For milk and fat requirement, however, there is a strong correlation to age, so the classes were considered separately, each class having its own mean and deviation. Curves 2, 3, and 4 were based on these class means and deviations. Curve

No. 2 is the mean of the population. Curve No. 3 is the plus deviation from the mean. *Curve No. 4 is a curve*



which was plotted to show what the requirements ought to be if the means, deviations and varying capacity of the different classes are taken into account. In plotting this curve it was necessary to consider the basis upon which the minimum requirements of this population ought to be placed.

The minus deviation point can not show what ought to be required of the class as a minimum, for such point would weight individuals inversely in proportion to their capacity. A greater deviation from the mean of the class

indicates here greater capacity for production of that class, and as the capacity for production of the class increases, so should the requirements increase. Therefore, the curve of minimum requirement should be represented as following the curve of plus deviation in character and should be in a minus direction from the mean.

In order to conform to these conditions some basis must be established for calculating the minus points of the curve, or, in other words, the minimum requirements for each class. The average deviation of the whole population seems to be the logical basis upon which the minimum requirement should be based, for by its use the whole curve may be lowered an amount corresponding to the average deviation of the whole population below the mean of the population. The average deviation from the mean of the whole population is 69.8 pounds of milk. If all classes are to be given the benefit of the average deviation the calculation should start from the point at which the means are at the maximum, which is about the six-year class. Hence the six-year class is allowed as the minimum requirement, the 69.8 pounds below the mean of the class and the requirements of the other classes are worked out from this point to conform, as said before, to the maximum deviation curve.

An inspection of these curves brings out the following points:

That the official requirements weight animals of an age from 18 to 21 months too heavily. The curve indicates that they are entitled to a reduction as great as for any other age. For the purpose of discouraging such early breeding, however, the requirements for this particular class should be prohibitive and they are.

That the production increases up to at least *six years* of age instead of five, which the Holstein-Friesian Association requirements set as the maximum age production.

That for this reason the 5- to 6-year-old animals and possibly the 7- to 8-year classes have an advantage over all other classes.

That a comparatively small number of animals made

the requirement after 9 years of age, hence by selection, only the best animals were retained, thus drawing the curve down almost to a straight line. The tendency of the curve, however, is to recede, showing that the animals of these ages should not be weighted as heavily as younger animals. A study of a number of representatives of the whole breed would be necessary to determine this point.

One of the most striking points shown by these data and one which substantiates the opinion of practical breeders of Holsteins, also brought out in the practical investigations of Eccles,[1] is the difference in production and capacity between 2- and 2½-year-old and 3-year-old cows. The difference in the means of the production between 2 and 2½ years was 18 pounds only, while between 2½ and 3 years it was 46 pounds, or a total of 64 pounds between the 2- and 3-year classes. Between the 3- and 4-year classes the difference is almost as great, being 56 pounds, but the deviation of the latter class is not quite as great as the former. This seems to indicate that the 3-year animal is still at a disadvantage by reason of its immaturity in growth and body development. That the average deviation of 2½-year class was 43 pounds while the 3-year class deviated 56 pounds is significant also and leads to the conclusion that at 2½ years of age the Holstein is still growing, and this, combined with the great strain of milk production, limits the capacity of the class.

It may be said by some that few 3- and 4-year-old animals are tested for advanced registry in comparison to two year olds and aged animals, and in consequence of this, only the best of the class make the requirements. This is not borne out by the data, the number in the 3-year-old class being second largest of all animals.

CURVES OF FAT PRODUCTION

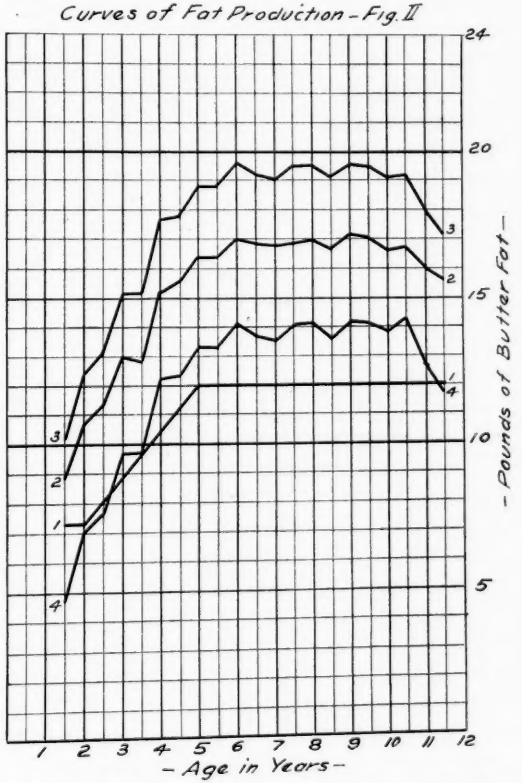
A study of the curves based upon the actual fat production of this population (Fig. 2) brings out a number

[1] Bul. No. 135, Missouri Agricultural Exp. Station.

of points, many of them corroborating those brought out in the discussion of the milk-production curves.

Owing to the variation of the weight classes in per cent. fat, the curves of milk production and fat production agree very well when compared with the Holstein-Friesian Association requirement curve.

The requirement curve in fat production (No. 4) crosses the Holstein-Friesian Association curve at a



greater age than that worked out for milk production. This would indicate that the classes up to $3\frac{1}{2}$ years produced milk containing a lower per cent. fat than the mean of the whole population. This is correct, as can be found

from the means of the classes. (See average per cents. of class means, Table V.) A similar condition obtains with the age classes after ten years. It would appear from this that mature cows give milk slightly richer than immature cows, or than old cows past 10 years of age.

A rather peculiar condition with reference to the fat production curve is shown in the mean results of the half-year ages up to the 6½-year class. Each half-year class advances but slightly, if at all, from its preceding year class, then there is a sudden drop to the next full-year class. The milk production curves indicate the same condition, though to a lesser extent, and as previously noted, the frequencies in these half-year classes are not more than 60 per cent. of the full-year classes. No good explanation is offered for this. It might be inferred that a cow freshening at 2½ years is not much better able to withstand the strain of milk production than a 2-year-old, and that this condition continues. However, in many respects this theory does not appear sound.

Attention is called again to the points of curve 4 for fat production given in Table IV. This curve is plotted for the purpose of showing what the requirements ought to be according to the performance of cows that have made records. The animals involved in this curve represent 45 per cent. of all the A.R.O. records that had been made up to the time of publication of Vol. 24, hence the

TABLE V

AVERAGE PER CENTS. FAT OF THE CLASSES

Age, Years	Per Cent.	Age, Years	Per Cent.
1½	3.28	7	3.51
2	3.27	7½	3.61
2½	3.47	8	3.58
3	3.49	8½	3.49
3½	3.24	9	3.59
4	3.55	9½	3.58
4½	3.48	10	3.69
5	3.58	10½	3.50
5½	3.56	11	3.47
6	3.58	11½	3.34
6½	3.54		

numbers are ample. First, the means of the classes of this population were plotted. Then their ability to deviate in a plus direction, or, in other words, to produce more fat as individual classes was taken into account. The class that had the maximum production and deviation ability was allowed, as a basis for its minimum requirement, the full average deviation of the population in a minus direction from the mean, and finally the other classes that could not produce as much and had not the ability to deviate as much as this maximum class, were allowed the full minus deviation of the population plus the difference in deviation ability between their particular class and the maximum class which forms the apex of the curve.

If these fundamental allowances are fair, impartial and accurate, the curve is accurate, and the only question that remains is whether or not it should alter the requirements of the Holstein-Friesian Association. If curve 4 touches the Holstein-Friesian Association curve at any point and does not coincide with it throughout, then the latter should be changed. It *does* touch it at both beginning and end, showing that all classes after the $2\frac{1}{2}$ years and up to $11\frac{1}{2}$ years have an advantage over the others. This advantage is greatest for the classes between $5\frac{1}{2}$ and 11 years of age.

The next consideration in connection with curve 4 is its application, and, when dealing with this, two things should be kept in mind; first, the practical, and secondly, the more concise and mathematical application. The practical application finds its expression in the endeavor of the Holstein-Friesian Association to make a uniform advance per day in the fat requirement for the seven-day test up to the age at which it was considered the maximum production was reached. Table VI compares the increase in the amount of fat required each year over that required in the previous year from two up to six years, with the increase in amount of fat that the year classes are *able* to produce as calculated from curve 4.

TABLE VI

Age, Years	H. F. A. Requirements		Curve 4 Requirements	
	Fat Increase, Yearly	Fat Increase, Daily	Fat Increase, Yearly	Fat Increase, Daily
2 to 3	1.6 lbs.	0.00438	2.70 lbs.	0.00740
3 to 4	1.6 "	0.00438	2.54 "	0.00696
4 to 5	1.6 "	0.00438	1.06 "	0.00290
5 to 6	0.0 "	0.0	0.79 "	0.00216

The table shows plainly that the daily increased requirement from 2 to 3 years should be 0.0074 instead of 0.00438, or 1.7 times as much. From 3 to 4 years should be 0.00696 instead of 0.00438, or 1½ times as much. From 4 to 5, 0.0029 instead of 0.00438, of nearly ½, and from 5 to 6 years, 0.00216 instead of no increase.

POPULATION NO. 2

The second population tabulated is that which began with Aaggie Grace No. 2618, H.H.B., as the primary ancestress, and consists of only 456 animals. Correlation tables 7 and 8 are omitted, but 9 and 10 are given, and show all the data necessary for comparison with the previous population. Of course, it must be borne in mind that the comparison can not be too exacting, for this population is altogether too few in numbers to secure smooth results especially when comparing classes. In fact, the class means and deviations, Table IX, included only the classes up to 9 years because of the low frequencies after that age. If Tables III and IV are compared with 9 and 10, a remarkable agreement is noticed throughout, especially in the essential points which have been discussed.

The correlation table for age to per cent. of fat is not shown, but the coefficients of this table may be seen in Table X. The correlation coefficient is so small that it may seem negligible, but Table V shows that even with a low correlation, important points might be brought out if the data are sufficient.

No endeavor will be made in this paper to enlarge on the exact mathematical application of these data. This

will be taken up later in connection with a further study of the two populations.

TABLE IX

CLASS MEANS AND DEVIATIONS OF POPULATION 2

Age, Years	Age to Pounds Milk		Age to Pounds Fat	
	Mean Pounds Milk	Average Deviation	Mean Pounds Fat	Average Deviation
2	286	47.3	10.35	1.65
2½	310	48.6	11.35	1.63
3	392	52.5	13.16	1.87
3½	403	53.5	14.22	1.97
4	413	60.0	14.30	2.20
4½	470	57.8	16.5	2.28
5	469	56.6	16.42	2.30
5½	484	41.4	16.27	2.15
6	504	66.4	17.23	2.26
6½	406	93.9	17.75	3.37
7	480	59.2	16.45	1.56
7½	471	47.0	17.22	2.14
8	468	60.7	16.27	2.48
8½	500	40.0	18.20	3.92
9	447	47.6	15.00	2.29

TABLE X

POPULATION COEFFICIENTS: POPULATION 2

	Age to Pounds Milk	Age to Pounds Fat	Age to Per Cent. Fat
Means	405.4 ±2.8	13.89 ±0.113	3.455±0.014
Standard Deviations	90.39 ±2.02	3.593±0.08	0.436±0.009
Correlation Coefficients N	0.592±0.02	0.581±0.02	0.08 ±0.031
Coefficient of Variability C	0.223±0.005	0.258±0.006	0.126±0.003
Regression Weights to Age	26.51 ±0.042	1.057±0.002	
Regression Age to Weight	0.022	0.546	

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SHORTER ARTICLES AND DISCUSSION

ŒNOTHERA NEO-LAMARCKIANA, HYBRID OF *O. FRANCISCANA* BARTLETT × *O. BIENNIS* LINNÆUS

Œnothera neo-Lamarckiana is a name which I propose for a synthetic hybrid that so closely resembles *O. Lamarckiana* De Vries that I do not believe systematic botanists could separate it from the latter by characters which would enter into a specific description. This does not mean that the hybrid is the exact counterpart of any particular line of *Lamarckiana* carried forward by the geneticists who are working with this form for it must be remembered that there are numerous biotypes of this species differing from one another in matters of greater or less detail, and that workers with œnotheras know that *Œnothera Lamarckiana* of systematic literature is a collective or polymorphic species, various forms of which can be isolated as biotypes in the experimental garden. In my studies of the *Lamarckiana*-like hybrids I am selecting towards the type known to us through the work of De Vries and through the seeds distributed by him.

The parents of my hybrids are *O. biennis* from the sand dunes of Holland and *O. franciscana* from California. I have given in a recent paper[1] the contrasting characters of these species together with descriptions of hybrids in the first and second generations. These parents were chosen after several years of search among the œnotheras for wild species that might be crossed with the hope of obtaining *Lamarckiana*-like types. In this connection my attention was first called to *franciscana* by Prof. Bartlett. In *biennis* and *franciscana* together are suggested all of the essential taxonomic characters of *Lamarckiana* and there seemed good reason to expect that among hybrids of the second and later generations would be found forms with combinations of characters approaching very closely to the peculiarities of *Lamarckiana*. In this respect my cultures now in the fourth generation have yielded results quite as satisfactory as I have hoped.

[1] Davis, B. M., "Hybrids of *Œnothera biennis* and *Œnothera franciscana* in the First and Second Generations," *Genetics*, I, 197–251, 1916.

The results will, I think, show that *Lamarckiana*-like forms of *Œnothera* may be synthesized by simple crosses between wild species provided the parent species are selected with care. I believe that as the isolation of *Œnothera* types proceeds a number of different crosses will be found to give similar results, but this is the first successful combination that I have been able to study experimentally. My earlier work[2] with *Œnothera grandiflora* Solander and certain American wild types was planned at a time when *grandiflora* on historical grounds seemed to be a more important type in relation to the problem of the origin of *Œnothera Lamarckiana* than it does at present. That work was not so successful as the later in producing *Lamarckiana*-like hybrids for the reason that the parent species did not have as favorable characters for the end in view.

In spite of the recent paper of De Vries,[3] to which I have replied[4] in brief, my conviction is unshaken that Lamarck's plant, grown in the botanical gardens of Paris about 1796, was a form of *Œnothera grandiflora* Solander and can not be identified with the *Lamarckiana* of De Vries's cultures. On this view *Œnothera Lamarckiana* Seringe must pass into the synonymy of *Œnothera grandiflora* Solander. Neither am I convinced that other specimens in the collections of the Muséum d'Histoire Naturelle in Paris, particularly sheets of André Michaux and Abbé Pourret, may be referred to *Œnothera Lamarckiana*. I believe that the plant with which we are concerned in the experimental garden had a later origin and must bear the name of De Vries as its sponsor. At present our first certain date of the progenitors of *Œnothera Lamarckiana* De Vries appears to be about 1860, when the seed firm of Carter and Company in London introduced the plant to the trade.

Both De Vries and Gates have accepted my suggestion that Carter and Company obtained their material of *Lamarckiana* from some English station and not from Texas, as they state. We have no evidence that *Lamarckiana* ever grew in Texas and to me there is no evidence that it was ever native to America.

[2] Davis, B. M., "Some Hybrids of *Œnothera biennis* and *O. grandiflora* that Resemble *O. Lamarckiana*," AMER. NAT., XLV, 193–233, 1911. "Further Hybrids of *Œnothera biennis* and *O. grandiflora* that Resemble *O. Lamarckiana*," *Ibid.*, XLVI, 377–427, 1912.

[3] De Vries, Hugo, "The Probable Origin of *Œnothera Lamarckiana* Ser.," *Bot. Gaz.*, LVII, 345–361, 1914.

[4] Davis, B. M., "Professor De Vries on the Probable Origin of *Œnothera Lamarckiana*," AMER. NAT., XLIX, 59–64, 1915.

On the other hand, various races of *Lamarckiana* are at present growing wild in a number of English localities, the best known stations being on sand hills of Lancashire near Liverpool. A conspicuous *Œnothera* flora was present in this region as early as the beginning of the nineteenth century, as shown by an account in Smith's "English Botany," 1806. There seems to be no reason why *Œnothera Lamarckiana* might not have arisen in such a locality as a hybrid of species introduced into England possibly through Liverpool as a port of entry. Thus we are dealing with dates of introduction or origin that are reasonably close to present times; attempts to associate *Lamarckiana* with very early introductions into Europe appear no longer to have important support.

It is necessary to bear in mind this historical setting, since it may seem to my readers very improbable that *Œnothera Lamarckiana* should have arisen as a hybrid between *franciscana,* a species of western America, and *biennis* of Holland, England and other European countries. There is, however, nothing improbable in the possible meeting at Liverpool, with its world-wide commerce, of species of *Œnothera* from far corners of the earth. Furthermore, I should be the last to suggest that the particular races or species which give my *neo-Lamarckiana* have been the actual parents of the strains of *Lamarckiana* cultivated by De Vries. To strike the identical parental lines of such an assumed hybrid would in the case of the œnotheras be a most extraordinary piece of luck. It is remarkable that my results have proved so satisfactory; I have no doubt that other species crosses may sometime be made which will give hybrids as close or even closer to *Lamarckiana.*

The line of *neo-Lamarckiana,* which I now have in the F_4 generation from the original cross, was derived from a single selfed plant in the F_2 (14.53*c*), which fell well within the range of variation given by De Vries for *Œnothera Lamarckiana.* A description will later be published of this plant together with an account of its progeny through successive generations when these have been carried along somewhat further. The F_3 generation gave very few *neo-Lamarckiana* types, but these were closer to the large-flowered forms of De Vries's cultures. This F_3 generation was grown from earth-sown seeds and incomplete germination may have been responsible for the small proportions of *neo-Lamarckiana,* 7 in a total of 291 plants. The F_4 generation

was grown this summer from what seemed to be the most promising plant of the F_3 (15.53*a*). This culture was from seed germinated in Petri dishes and was complete, since the residue of ungerminated seeds were empty of contents. From 764 seed-like structures 668 seedlings appeared, but there was at once a large mortality among weaklings most of which were unable to free their cotyledons from the seed coats. Only 558 seedlings lived to be potted and a further mortality reduced the number that was set out in the garden to 549. Of these plants 198 as rosettes presented characters of *Lamarckiana* while 351 developed rosettes for the most part with narrower leaves suggestive of *franciscana*. All of the shoots from the 198 *Lamarckiana*-like rosettes have shown *Lamarckiana* characters of foliage, inflorescence, and flowers but about one fourth of the plants seem likely to persist this summer as rosettes. The group of *neo-Lamarckiana* in the F_4 generation is therefore large constituting about 36 per cent. of the total number of plants in the culture.

In the group of *neo-Lamarckiana* there is some variation, but the best plants are so close to the *Lamarckiana* of De Vries that I can only distinguish them by small plus or minus expressions of a few characters. Thus the central shoot is not so strongly developed proportionally to the side branches. The leaves are a little broader. Sepal tips do not spread so widely. Buds may not be quite so stout. The pubescence is somewhat heavier over certain portions of the plants. Time will tell whether even these small differences can be eliminated by judicious selection through succeeding generations.

It is of course not enough for critical bearing on De Vries's interpretation of the behavior of *Lamarckiana* that a hybrid should be synthesized taxonomically similar to it. Such a hybrid must also show a behavior parallel to *Lamarckiana* in its essential features. The two striking peculiarities in the breeding habits of *Lamarckiana* are (1) its ability to produce two types (twin hybrids) in the F_1 when mated to certain other species, and (2) its peculiarity of throwing through successive generations the same types of "mutants" in small, fairly constant proportions. Late in the season of 1915 reciprocal crosses were made between *neo-Lamarckiana* (15.53*a*) and plants of *biennis* and *biennis* (Chicago), forms which De Vries has used in his studies on twin hybrids from *Lamarckiana*. The conditions were not favorable for the technique of crossing and I am repeating the experi-

ments this year. However, I obtained from the cross *biennis* × *neo-Lamarckiana* two distinct classes of plants, (1) a narrow-leaved, smaller-flowered type with heavy pubescence and red papillæ (109 plants), and (2) broad-leaved forms, some larger-flowered, with a much lighter pubescence and few or no red papillæ (11 plants). Also, the cross *neo-Lamarckiana* × *biennis* (Chicago) gave two clearly defined classes distinguished at a glance by their size and foliage, (1) tall and narrow-leaved (64 plants), and (2) shorter and broad-leaved (11 plants). These crosses appear to have given twin hybrids and it should be said that the two groups were recognized and separated when the plants were in the rosette stage and that they consistently presented differences throughout all stages of their development. I shall from time to time make further studies of this behavior with different generations of *neo-Lamarckiana*. If *biennis* and *biennis* (Chicago) are pure species (a matter not yet established) this behavior would indicate that *neo-Lamarckiana* develops at least two classes of fertile gametes for both pollen and ovules. It thus seems probable that the behavior of *neo-Lamarckiana* when crossed to other species of *Œnothera* will parallel that of De Vries's *Lamarckiana* and thus support the view of several critics of the mutation theory that *Lamarckiana*, because it gives twin progeny in the F_1 of certain species crosses, must be itself a hybrid, producing different classes of gametes.

With respect to the ability of *neo-Lamarckiana* to throw "mutants" a most interesting situation is presented by its behavior this summer in the fourth generation. We have noted that a sowing of 764 seed-like structures gave 668 seedlings of which 198 developed as rosettes or mature plants into *neo-Lamarckiana*. Of the remaining 470 seedlings (668–198) only 351 lived to produce rosettes, a much larger group, however, than that containing the parent type, *neo-Lamarckiana*. We have then in the fourth generation *neo-Lamarckiana*, an impure or hybrid species, reproducing itself from at least 26 per cent. of its seeds. The exact percentage can not be told, for we do not know whether any plants of *neo-Lamarckiana* were among the 119 seedlings that died. In throwing a large progeny of a type very different from the parent F_3 plant, *neo-Lamarckiana* in the F_4 exhibited a behavior with strong resemblance to what Bartlett has described as "mass mutation." The types included a number of dwarf forms, but most of the plants resembled *franciscana*,

although generally stronger, larger-leaved, and with considerable variation in flower size. It should be noted that no forms similar to the parent *biennis* were present; this type of segregate seemingly is either not produced or appears but rarely.

The conditions of sterility in *neo-Lamarckiana* are likely to bear directly on the peculiarities of its behavior in comparison with that of De Vries's plant. My hybrids agree with *Lamarckiana* in having pollen about one half sterile, but the F_3 parent plant of this year's cultures showed seeds 87 per cent. fertile while the seed fertility of *Lamarckiana* is much lower, being reported by De Vries in extensive experiments as from 34.5–46 per cent. and for two lines of mine running in tests 26–30 and 32–36 per cent., respectively. The variation noted by De Vries is believed by him to depend upon whether or not the plants are heavily manured. The question at once arises may not the mass variation of *neo-Lamarckiana* in the F_4 be correlated with its very much higher seed fertility? What would happen if *neo-Lamarckiana* should develop a greater degree of seed sterility or if some lines should be segregated with seed sterility approaching that of De Vries's *Lamarckiana?* Would the plants eliminated come from among the *neo-Lamarckianas* or would they come from the assemblage of variants from this parent type? Should they come from the variants, as seems to me probable since *neo-Lamarckiana* is a sturdy plant, then a condition might be reached where the variants would appear rarely or in small proportions and this would parallel exactly the present behavior of De Vries's *Lamarckiana* in throwing its "mutants." I shall watch intently for indications in my cultures of increased seed sterility and among my plants of *neo-Lamarckiana* select steadily towards the higher degree exhibited by *Lamarckiana.* It is interesting that the last stages in the experimental synthesis of a *Lamarckiana*-like hybrid should be concerned chiefly with selection towards a definite degree of seed sterility.

Œnothera neo-Lamarckiana illustrates clearly my concept of an impure species of *Œnothera.*[5] It is a plant that breeds true in a proportion of its offspring but is heterozygous since it develops varied types of gametes as proved by the assemblage of offspring which differ sharply from the parent plant, and further indicated by its behavior in producing twin hybrids. The facts of a high degree of pollen sterility (about 50 per cent.) together

[5] Davis, B. M., "The Test of a Pure Species of *Œnothera,*" *Proc. Amer. Phil. Soc.,* LIV, 226–245, 1915.

with a certain amount of seed sterility indicate the probability that other classes of gametes are eliminated or fail to function and that possibly certain types of zygotes may be formed which are unable to live. *Œnothera neo-Lamarckiana* therefore shows itself to be impure or heterozygous because it develops different types of gametes even though the plant when selfed reproduces itself in a fairly large proportion of its progeny. This behavior seems to me quite the same in principle as that of De Vries's *Lamarckiana,* the only difference being that the total number of individual variants thrown by *Lamarckiana* is much smaller than those *at present* thrown by *neo-Lamarckiana.* However, as has been noted, the seed sterility of *Lamarckiana* is very much higher than that of *neo-Lamarckiana* in the F_4 generation, and it is my working hypothesis that this fact is at least partly responsible for the smaller numbers of variants produced by the former.

Œnothera Lamarckiana of De Vries's cultures seems to me best interpreted as an impure species producing regularly because of its heterozygous or hybrid nature a number of classes of gametes relatively few of which, because of the extensive sterility, both gametic and zygotic, are able to form viable seeds different from those that reproduce the species. *Œnothera Lamarckiana* breeds true in its high degree because only the gametic combinations that reproduce *Lamarckiana* survive the mortality visited on most of the gametes and zygotes. By this view *Lamarckiana* is very much the reverse of a representative pure species which De Vries has assumed it to be and its "mutating habit" is the result of its hybrid origin and heterozygous nature rather than a spontaneous expression of homozygous germ plasm. The fact that the same types of "mutants" from *Lamarckiana* are produced by successive generations in fairly stable proportions indicates that their differentiation lies in the mechanism of segregation in heterozygous germ plasm rather than in a sporting tendency (mutation) which would be expected to express itself in ever-varying ways and degrees.

It is worth noting how different is the conception, here expressed, of the constitution of a pure species from the view formerly and probably now very generally held. Formerly a species was considered pure if it bred true. Now we believe that a species may be impure and still breed very largely or even wholly true if a degree of sterility is present sufficient to render

abortive or infertile all types of gametes or zygotes that may be produced except the ones which carry forward the heterozygous line. The test of a pure species is then not that it should breed true (that is a corollary), but that it should produce gametes uniform except as they may differ with respect to the factors for sex characters.

In laying such great stress on the phenomena of gametic and zygotic sterility so very extensively present in the genus *Œnothera* it must not be supposed that we have as yet established the degrees to which sterility may be genetic in its character or to what extent it may be of a physiological nature. Only the sterility that has as its cause the failure of the reduction divisions to produce fertile gametes or the failure of the gametes to conjugate freely can properly be of a genetic nature. There is probably also a type of sterility due to physiological causes, as perhaps malnutrition, and this might affect gametes and zygotes which under favorable conditions would be fertile. We are very far from an understanding of the causes of sterility in *Œnothera*, to what extent cytological or to what degree physiological, and it would at present be most unsafe to carry lines of speculation very far in this field as regards the material under consideration.

Professor De Vries has expressed strongly a belief in the futility of my attempts to synthesize a *Lamarckiana*-like hybrid, taking the stand that unless the parent stock is known to be stable mutability might be inherited from one or both of the parent species, or that variants, the result of a cross from impure stock, might be mistaken for mutations. As a matter of fact *Œnothera biennis* is known to be unstable, producing a small series of "mutants," while *O. franciscana* has not been tested for its purity. Apparently Professor De Vries and I are working from assumptions that are far apart. The inheritance of a mutating habit such as that claimed for *Lamarckiana* would mean to me the inheriting of a heterozygous germ plasm running back to some hybrid origin. To me phenomena such as is exhibited by *Lamarckiana* in throwing its "mutants" indicates in itself the probability of heterozygous germ plasm. If this behavior is to be presented as evidence of mutation the purity of *Lamarckiana* must be established beyond all reasonable doubt and this in my opinion has not been shown. The tests of cross breeding, when twin hybrids result, and the very high degrees of gametic and zygotic sterility strongly indicate genetic im-

purity. And back of this is an obscure history for the material with no evidence it seems to me that *Lamarckiana* was ever present as a native species of any flora. The chief value which the study of my *Lamarckiana*-like hybrid may have for the problem of the origin and status of *Œnothera Lamarckiana* is likely to be a clearer understanding of how an obviously impure species, *neo-Lamarckiana*, may arise, a species which seems likely to present a breeding behavior parallel to that of *Lamarckiana*, and most important of all the significance of sterility in the working out of these results. It appears to me a matter of no vital importance to the status of a hybrid whether its parents are pure or impure. If markedly impure the problem of analysis for future generations merely becomes the greater. Since no species of *Œnothera* has as yet passed the tests for a pure species, we are at present in all of the *Œnothera* work talking of an abstraction when this concept is considered.

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August, 1916

STATISTICAL STUDIES OF THE NUMBER OF NIPPLES IN THE MAMMALS

IT is perhaps not unnatural that a subject of such fundamental interest as that of the nourishment of the young in the mammals should have attracted the attention of observers from the time of the Greek philosophers. It is only within the last few years that attempts have been made to solve various problems by the application of the statistical method to series of quantitatively recorded data.

The materials may be divided for convenience of review.

TYPE, VARIATION AND CORRELATION IN NUMBER OF MAMMÆ

The statement made by Parker and Bullard,[1] on the basis of their splendid series of data for swine, that the standard deviation of the number of nipples is 0.6906 in the males and 0.7905 in the females at once arouses the suspicion of a biometrician. The constants actually are:

[1] Parker, G. H., and C. Bullard, "On the Size of Litters and the Number of Nipples in Swine," *Proc. Amer. Acad. Arts and Sci.*, **49**: 399–426, 1913.

	For Males	For Females
Mean	12.4365 ± .0182	11.9077 ± .0159
Standard Deviation	1.4800 ± .0128	1.2803 ± .0112
Coefficient of Variation	11.901 ± .105	10.752 ± .096

Thus instead of the females being "over 14 per cent. more variable than the males" they are in absolute terms actually .1997 ± .0175, or over 13 per cent., *less* variable. Relative variability as measured by the coefficient of variation is 1.149 ± .142 per cent. lower in the female that it is in the male. This lower variability of the female is also quite in evidence if the materials be split up into groups with regular and irregular arrangement of the nipples. Thus:

For "Regular" Class

Males	$\sigma = 1.485 \pm .017$,	C. V. = 12.03 ± .14
Females	$\sigma = 1.315 \pm .021$,	C. V. = 11.16 ± .13
Difference	0.170 ± .027,	0.87 ± .19

For "Irregular" Class

Males	$\sigma = 1.461 \pm .020$,	C. V. = 11.61 ± .16
Females	$\sigma = 1.210 \pm .016$,	C. V. = 10.02 ± .14
Difference	0.251 ± .026,	1.59 ± .21

However measured, the variability of the number of nipples in the female is always significantly less, *not greater,* than in the male.

Furthermore a rather noteworthy sexual differentiation seems so far to have escaped notice. The mean number of nipples for male pigs is in all cases higher than that for female pigs. Thus:

All Pigs

Males	12.4365 ± .0182
Females	11.9077 ± .0159
Difference	0.5288 ± .0242

Classified as Regular		Classified as Irregular	
Males	12.3425 ± .0233	Males	12.5833 ± .0234
Females	11.7849 ± .0214	Females	12.0777 ± .0232
Difference	0.5576 ± .0316	Difference	0.5056 ± .0330

In all cases the males have on the average more nipples than the females. The regularity of the differentiation is brought out

by the accompanying table in which actual values have been reduced to *per mille* frequencies. Pigs with 12 nipples or fewer are preponderantly females; pigs with 13 nipples or more are preponderantly males.

Number of Nipples	Male	Female	Difference
8	.0	.3	+ .3
9	.6	1.7	+ 1.1
10	90.6	143.7	+ 53.1
11	162.7	217.9	+ 55.2
12	332.0	370.0	+ 38.0
13	167.6	154.5	— 13.1
14	163.4	82.8	— 80.6
15	49.3	20.0	— 29.3
16	29.8	7.1	— 22.7
17	3.0	1.7	— 1.3
18	1.0	.3	— .7
	1000.0	1000.0	

The correlation between the number of nipples on the two sides are:

Males	.6359 ± .0073
Females	.5419 ± .0088
Difference	.0940 ± .0114
All Pigs	.6063 ± .0055

The correlations are fairly high. Those for males seem to be slightly larger than those for females.

CORRELATION BETWEEN THE NUMBER OF THE YOUNG IN THE LITTER AND THE NUMBER OF MAMMÆ IN THE DAM

The relationship between the number of young per litter and the number of mammæ in the female has at various times aroused considerable interest. As Pearl[2] has pointed out, two kinds of correlation are to be recognized. First, interracial correlation, that between the mean size of the litters and the mean number of mammæ in the females of a series of races or species. Second, intraracial correlation, that between the number of mammæ in an individual mother and the number of young that she bears.

It is the rather obvious interracial correlation that has given rise to such statements as that of Gegenbaur: "Die Zahl der Zitzen steht in inniger Beziehung zur Menge der Jungen." It

[2] Pearl, R., "On the Correlation between the Number of Mammæ of the Dam and Size of Litter in Mammals. I. Interracial Correlation," *Proc. Soc. Exp. Biol. Med.*, 11: 27–30, 1913.

was the problem of intraracial correlation with which Alexander Graham Bell[3] was dealing when he studied the fertility of the multi-nippled race of sheep at Beinn Bhreagh.

Notwithstanding the simplicity of the biological problem a certain amount of confusion seems to have arisen. Thus Parker and Bullard (*loc. cit.*) state:

> It is the chief object of our paper to discuss the relation of the size of litters to the number of nipples in the domesticated swine, *Sus scrofa Linn.*

But instead of determining the correlation between the number of teats of the sow and the number of her young they have actually calculated the relationship between the number of siblings in the litter in which a pig was born *and the number of nipples which she herself possesses!* Surely it should not require specialization in animal behavior to convince one that the teats which are of real service to a young pig are not its own, but those of its mother!

Pearl[4] has quite correctly determined the correlation between the number of nipples in the individual mothers and the number of young in their litters. This he finds to be very low,[5] $r = 0.195 \pm .086$.

It is rather difficult to agree with Pearl in his statement that

> It would seem, *a priori*, that natural selection should have operated to bring about a high correlation, both intra- and inter-racial between these two variables, size of litter and number of mammæ in the dam.

There seems no reason whatever to suppose that natural selection would tend to produce a correlation between the number of mammæ in the mother and the size of her litters *within a race, providing it has produced an average number of nipples suffi-*

[3] Bell, Alexander Graham, *Science*, N. S., 9: 637–639, *pl. 5*, 1899; *loc. cit.*, 19: 767–768, 1904; *loc. cit.*, 36: 378–384, 1912.

[4] Pearl, R., "On the Correlation between Number of Mammæ of the Dam and Size of Litter in Mammals. II. Intraracial Correlation in Swine," *Proc. Soc. Exp. Biol. Med.*, 11: 31–32, 1913.

[5] Wentworth (*Jour. Agr. Res.*, 5: 1148, 1916) records another very low coefficient on unpublished data, but does not state specifically whether it is between the number of mammæ of the mother and the number of her young as in Pearl's series, or between the number in a litter (weighted with their own number) and number of nipples in the individual pigs, as in the series of Parker and Bullard.

ciently large to maintain the race.[6] On the contrary, any theory of ontogeny or phylogeny which demands the existence of a mechanism to provide an embryo pig with the particular number of nipples which would agree closely with the number of young she may be destined to bear as an adult would seem to be not merely cumbersome, but unnecessarily teleological. Since male pigs have more mammæ than females, the cost to the organism is apparently not prohibitive! What one should expect as the result of the action of natural selection would, therefore, not be the development of a regulative mechanism to provide the mother with a number of nipples in close agreement with the size of her future brood, but the development of a number of nipples sufficiently large for the needs of the race.

Pearl's own data show only 7 out of 57 "disadvantageous" combinations, and the table as it stands takes no account of early deaths.[7] Furthermore, his series is small, only 57 individuals, and apparently hardly typical of swine as a class. Parker and Bullard on the basis of a thousand litters show that the (empirical) modal number of nipples is twice the modal number of young, and that the average number of nipples is much more nearly twice the number of young than in Pearl's short series. Thus the data of both Pearl and Parker and Bullard indicate in the words of the latter authors that "disadvantageous combinations in which the number of young pigs outrun the provision for

[6] Natural selection can not be expected to accomplish more for the development of any character than to bring it to and maintain it at a stage of development necessary for the survival of the species in competition with others. That correlation between the number of the young and the number of nipples is not necessary under conditions of domestication is shown by the classic observations of Minot on the guinea pig (*Jour. Phys.*, **12**: 103, 1891) in which he pointed out that in his studies 143 litters showed a variation of from 1 to 8 in the number per litter, with a model frequency on 2 and an average of 2.5, although the number of developed mammæ is two.

That the number of young born may regularly exceed the number of nipples in a species persisting under natural conditions is shown by the recent studies of Hill and O'Donaghue on the marsupial *Dasyurus viverrinus* (*Quart. Jour. Micr. Sci.*, N. S., **59**: 133–173, 1914) in which they have shown that a remarkable number of eggs are discharged from the ovary at each ovulation and that as a rule more young are borne than can possibly survive because of the limited accommodation of the pouch.

[7] Unfortunately trustworthy figures showing directly the mortality of new-born or recently born pigs seem not to be available. That such mortality is considerable is indicated by certain of the figures given for another purpose by Evvard.

milk, cannot be of frequent occurrence.'' The development of just such a ''factor of safety'' and not the origination of an intraracial correlation is, as emphasized above, just what one would expect of natural selection.

Natural selection, if operative, should, however, bring about an interracial correlation, and this is exactly what observant biologists have always noted and Pearl has expressed statistically by the value $r = .594 \pm .046$, with non-linear regression—a value distinctively higher than that for the intraracial relationship. Thus, as far as they go, these observations instead of evidencing against natural selection, actually show the very conditions to exist which might be expected as the result of the action of this factor of organic evolution.

Inheritance of Number and Arrangement of Nipples in Swine

Attempts at the Mendelian analysis of inheritance of number and arrangement of mammæ in swine have been made by Wentworth,[8] who has suggested that the presence of rudimentary nipples is a sex-limited,[9] sex-linked,[10] or sex-limited[11] character. His final stand is that the pair of rudimentaries posterior to the inguinal pair behave as a Mendelian unit character in heredity, but that somatically it develops in males, which are *RR* or *Rr*, but in the females only when they are *RR*, where *R* indicates the presence and *r* the absence of the factor for rudimentaries.

It is interesting to return to the sexual dimorphism with respect to number of mammæ demonstrated above on the basis of Parker's and Bullard's splendid series of data and to consider it in connection with the hypothesis advanced by Wentworth.

Pearson many years ago showed[12] that with continued random mating the distribution in any generation subsequent to an original random pairing of *RR* and *rr* individuals is

$$\tfrac{1}{4}RR + \tfrac{1}{2}Rr + \tfrac{1}{4}rr.$$

[8] Wentworth, E. N., ''Inheritance of Number of Mammæ in Swine,'' Rep. Am. Breed. Ass., **8**, 1912.

[9] Wentworth, E. N., ''Another Sex-limited Character,'' *Science*, N. S., **35**: 986, 1912.

[10] Wentworth, E. N., ''Sex-linked Factors in the Inheritance of Rudimentary Mammæ in Swine,'' *Proc. Iowa Acad. Sci.*, **21**: 265–268, 1914.

[11] Wentworth, E. N., ''Rudimentary Mammæ in Swine a Sex-limited Character,'' *Science*, N. S., **43**: 648, 1916.

[12] Pearson, K., *Phil. Trans. Roy. Soc. Lond., A*, **203**: 59–60, 1904.

Both Pearl[13] and Jennings[14] have followed him in this point. If the thousand litters studied by Parker and Bullard come from a population homozygous and heterozygous with respect of a pair of rudimentary nipples in the 1:2:1 proportion and mating at random,[15] then three out of four males as compared with one out of four females should, if Wentworth's hypothesis be correct, show the pair of rudimentaries. Thus the average number of mammæ in the males should be 1 higher than in the females. As a matter of fact it is .529 ± .024 higher.

Further discussion on the basis of the present data would of course be idle.

In his largest paper Wentworth[16] has presented data which indicate sensible parental and grandparental correlations for number of mammæ. In view of the irregularity of the frequency distributions due to the modes on the even numbers and the smallness of the series, as well as the fact that the number of boars was very limited, little weight is to be given to the exact numerical values of his coefficients.

A more detailed analysis of the extensive series of data collected by Parker and Bullard may throw considerable light upon the problem of inheritance. The results must be expressed in terms of fraternal or sororal correlation. Those who are so obsessed with Mendelian theory that they are unwilling to learn anything about a series of data for which their method fails, should discontinue the reading of this review at this point.

Correlation between the number of nipples in siblings may be very readily found by means of intra-class correlation formulæ[17] involving first and second moments for the individual classes (litters).

Let x_m be the number of nipples in a male, x_f the number of nipples in a female pig, n_m the number of males and n_f the number of females in a litter of $n_m + n_f = n$ individuals. Let Σ denote summation within the litter and S a summation for litters. For any litter the moments are therefore $\Sigma(x_m)$, $\Sigma(x_m^2)$, $\Sigma(x_f)$,

[13] Pearl, R., AMER. NAT., 47: 606–609, 1913.

[14] Jennings, H. S., *Genetics*, 1: 64, 1916.

[15] Random mating of course applies only to the particular character in question, which is one which would hardly be consciously selected by any breeder.

[16] Wentworth, E. N., "Inheritance of Mammæ in Duroc Jersey Swine," AMER. NAT., 47: 257–278, 1913.

[17] Harris, J. Arthur, *Biometrika*, 9: 446–472, 1913.

$\Sigma(x_f^2)$. Since in a symmetrical intra-class correlation table the variates are weighted in an $(n-1)$-fold manner the fraternal correlation for males is given at once by direct summation from the data table of Parker and Bullard by the formula, written for simplicity in an entirely unreduced form,

$$r = \frac{\dfrac{S[\Sigma(x_m)]^2 - S\Sigma(x_m^2)}{S[n_m(n_m-1)]} - \left(\dfrac{S[(n_m-1)\Sigma(x_m)]}{S[n_m(n_m-1)]}\right)^2}{\dfrac{S[(n_m-1)\Sigma(x_m^2)]}{S[n_m(n_m-1)]} - \left(\dfrac{S[(n_m-1)\Sigma(x_m)]}{S[n_m(n_m-1)]}\right)^2},$$

or substituting actual values

$$r_{x_{m1}x_{m2}} = .323 \pm .019.$$

Apparently complex, the formula is really on closer inspection very simple indeed.

One altogether similar for the females gives the sororal correlation

$$r_{x_{f1}x_{f2}} = .373 \pm .018.$$

Thus the correlation for the females is $.050 \pm .026$ higher than that for the males.

For the cross correlations, that between number of nipples borne by male and female pigs of the same litter, the constant is given by

$$r = \frac{\dfrac{S[\Sigma(x_m)\Sigma(x_f)]}{S(n_m n_f)} - \dfrac{S[n_f\Sigma(x_m)]}{S(n_f n_m)} \times \dfrac{S[n_m\Sigma(x_f)]}{S(n_m n_f)}}{\sqrt{\dfrac{S[n_m\Sigma(x_f^2)]}{S(n_m n_f)} - \left(\dfrac{S[n_m\Sigma(x_f)]}{S(n_m n_f)}\right)^2}\sqrt{\dfrac{S[n_f\Sigma(x_m^2)]}{S(n_f n_m)} - \left(\dfrac{S[n_f\Sigma(x_f)]}{S(n_f n_m)}\right)^2}}$$

or

$$r_{x_m x_f} = .287 \pm .020,$$

a value apparently distinctly lower than that for either males or females alone.

If the correlation between the siblings be determined *irrespective of sex* the value is

$$r = \frac{\dfrac{S[\Sigma(x)]^2}{S[n(n-1)]} - \left(\dfrac{S[(n-1)\Sigma(x)]}{S[n(n-1)]}\right)^2}{\dfrac{S[(n-1)\Sigma(x^2)]}{S[n(n-1)]} - \left(\dfrac{S[(n-1)\Sigma(x)]}{S[n(n-1)]}\right)^2},$$

where the n and x without subscripts denote number of individuals per litter and number of nipples per individual, without reference to sex. Numerically

$$r_{x1x2} = .305 \pm .019.$$

This is lower than the relationship for either of the sexes individually considered, just as one might have predicted on *a priori* grounds from the low value of the cross correlation and from the differentiation in the number of mammæ in male and female pigs.[18]

The correlation coefficients here given show that there is a very material degree of resemblance with respect of nipple number in pigs from the same litter.[19] Indeed the correlation is about one third of the maximum value. Such correlation can be due only to differences in intra-uterine environment or to a strong inheritance of nipple number. The latter seems by far the more probable explanation.

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[18] Harris, J. Arthur, "On Spurious Values of Intra-class Correlation Coefficients Arising from Disorderly Differentiation within the Classes," *Biometrika*, **10**: 412–416, 1914.

[19] These values of the fraternal correlation will be but slightly influenced by the weighting of the individuals in the determination of the correlations, since nipple number is but slightly correlated with number in the litter.